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ISONING

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ZNIKOFF*

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INTRODUCTION[1]

Lead intoxication is not only the most common poisoning in industry, but also is derived from such diverse non-industrial sources as water supplies, cosmetics and drugs, and home-made beverages It is a preventable disease which may be considered from at least three points of view. In industry the necessity of preventive measures has long been realized and much has been written in regard to methods to reduce the lead hazard by improving conditions of work The legal aspect of the disease has been well summarized recently by Miss Hutton (222) and by Cantineau (65) and no further review is necessary here The biological problems involved in lead poisoning are of much scientific and practical interest This monograph deals with this aspect of the disease and discusses in some detail recent work which was undertaken to help explain the reactions produced by lead in the body

[1] The funds for this work were given to the Harvard Medical School by the National Lead Institute We are indebted to Drs Albert Key and Hermann Blumgart, and also to Drs A V Bock, J W S Brady, E J Cohn, Wm A Hinton, Chester Jones, and Misses J Hendry and A Hopkins for assistance in various portions of the experimental work, to Misses Charlotte Shaw Mary Morrison, Agnes Peltz, Helen Tracy, and Mrs Marion Frankfurter for technical aid and to Miss Martha Taylor for invaluable aid in preparing this manuscript To Dr C K Drinker, in whose laboratory our work was done, to Dean David Edsall, and to Prof A B Lamb and Dr Alice Hamilton, we are grateful for valuable aid and counsel

No attempt has been made to cover fully the literature of the subject Three thousand references were collected and published by Else Blansdorf in 1922 (38) and our files contain more than 2200 Only those references which bear directly upon the discussion are included here, although we have tried to summarize and include all the pertinent literature in order to obtain a fair perspective of present knowledge

The problems have been approached from their physiological and chemical aspects in order to obtain explanation, wherever possible, of the mechanisms involved in the production of the various conditions associated with the disease The first portion of the monograph is therefore composed of a consideration of the chemical problems associated with lead in the organism It includes methods of analysis and a description of the reactions of lead in the body during absorption, storage, and excretion A summary of the literature of the gross and microscopic pathology follows and leads to a discussion of the various toxic manifestations of plumbism in the light of the data bearing on the physiology of their production Finally, the clinical picture is given and an outline of the methods of treatment At the end is a summary of the present industrial situation very kindly contributed by Dr Alice Hamilton

I HISTORY

Even in ancient times the disease which is now known as lead poisoning was recognized As both Tanquerel des Planches (453) and Alderson (5) have very fully outlined the ancient history of lead poisoning, only a very brief statement will be included here The many symptoms of plumbism were noted long before they were ascribed to the action of lead Gradually, however, physicians came to appreciate the cause of these disturbances and the syndrome was designated "saturnism," for lead was "called saturn by the alchemists because it absorbs and devours, so to speak, all other imperfect metals in its scorification" (117) For many years only clinical descriptions appeared in the literature, and it was not until the early part of the nineteenth century that experimental investigation of the action of lead in the body was begun This has continued until the present day, and recently has been supplemented by attempts to improve

industrial conditions Consequently the incidence of plumbism has been markedly reduced.

Hippocrates (370 B C) was probably the first of the ancients to recognize lead as the cause of symptoms, at least he describes a severe attack of colic in a man who extracted metals The relationship of constipation, abdominal pain, and pallor to the action of lead on the body was also observed by Nicander in the second century B C This author also mentioned lead palsy for the first time. But probably not until the first century A D was the true significance of lead appreciated Dioscorides is quoted by Alderson as saying that "the drinking of litharge (red lead) causes oppression to the stomach, belly, and intestines, with intense wringing pains, sometimes it even wounds the intestines by its severe pressure, it suppresses the urine, while the body swells and acquires an unsightly leaden hue" He also speaks of paralysis and delirium as consequences of ingestion of lead. Many years later, about 1000 A D , Avicenna recommended the use of violent purgatives in doses graded according to the severity of the attack for a special form of colic which must have been caused by lead Crato who lived about 1600, definitely attributed the colic which was common in Moravia and was known to end in paralysis to the use of "falsified" wine; but it was not until 1656 that paralysis was definitely recognized as an effect of lead Stockhusen (444) then published a remarkable treatise in which he recognized that lead poisoning followed absorption of lead dust He also tried to determine which muscles of the arm are commonly paralyzed

In 1616 Citois (81) a physician of Poitiers, also mentioned bad wine which had been treated with lead as the cause of colic, and described the disease (*Colica Pictonum*) excellently although he attributed the disturbance chiefly to the acid of the wine Tronchin (467) pointed out in 1757 the relationship between this type of colic and the disease common among lead workers In 1745 Huxham (223) described a disease appearing in Devonshire, England, the symptoms of which were identical with true lead colic This was later (1767) traced by Sir George Baker to the lead employed in the manufacture and storage of cider In Spain a similar type of colic of similar etiology was described in 1796 by Luzuriaga (271). Shortly after this, in the beginning of the nineteenth century, several brilliant

clinical descriptions appeared Grisolle (178) described colic, lead line, and particularly encephalopathy very accurately, and three years later the epoch-making book by Tanquerel des Planches was published In this the clinical aspect of the disease is quite perfectly and completely outlined and most of the acute signs of lead poisoning are mentioned In spite of the fact that they laid emphasis on the lead line, this sign has come to be known as the Burtonian line because of a description published by Burton (59) in 1840 Since Tanquerel's time little has been added to our clinical knowledge of lead poisoning except by Déjérine-Klumpke (92) who wrote an excellent treatise on the lead palsies Several good discussions of the early differential diagnosis of plumbism have appeared recently

Experimental study of the disease was apparently started in 1814 by Orfila (344) who administered lead intravenously and by mouth He observed that its toxic action was greater after oral than after intravenous administration, and also that it was not a very poisonous metal Grisolle and Tanquerel also did some experimental work, though their contributions in this line were not striking They studied the toxicity, paths of entry and distribution of lead in the organism as well as the clinical picture of poisoning in animals Since their time many investigations have been carried out As these are reported in the chapters which follow, they need not be re-summarized here, but it should be stated that they have dealt with the modes of absorption and excretion of lead, and to a slight extent with an explanation of the mechanism of its action in the body Although some work has been centered upon the problem of palsy, most interest has been given to the obvious changes caused by lead in the blood Between 1865 and the beginning of the present century attention was focused upon the pathology of lead poisoning, but this produced very little new fundamental knowledge More recently, modern methods of histological staining have enabled some slight advance to be made in studies of the pathology of encephalopathy, but, on the whole, the most significant additions to an understanding of the disease have been derived from investigations of the localization of lead in the tissues

Another type of investigation which has proved of great value in lowering the incidence of lead poisoning has been a study of public

and industrial hygienic conditions This has resulted in the installation of various protective measures which have greatly reduced the incidence of toxic manifestations Probably the first public health work of this type was the elimination of the colic of Poitiers by preventing contamination of wine In 1860, Lefèvre (251) pointed out that the dry colic observed on ships and in the French colonies was caused by lead, and insisted that precautions be taken As a result the colic largely disappeared. Most of such work, however, has been done within the last twenty-five years, for the most part by Sir Thomas Oliver (337) and Legge and Goadby (252) in England, Meillère (293) in France, Teleky (455–459) in Germany, and Alice Hamilton (187–195) in America Throughout these countries the passage of many regulations to safeguard workers and give them compensation for injuries has resulted Both Cantineau (65) and Hutton (222) have recently summarized well the present legislative situation

Requirement of prophylactic measures and modern knowledge of the action of lead have greatly reduced the incidence of industrial lead intoxication, particularly within the last ten years. With proper precautions it should be possible to control lead poisoning completely, and thus avoid the need of eliminating the use of lead in industry.

Part I. Chemistry

II THE DETECTION AND ESTIMATION OF LEAD IN ORGANIC MATERIAL

The value of a satisfactory method for the detection of lead has been recognized for centuries, and various reactions have been suggested as the basis for suitable tests Because of the general use of metallic lead and later of litharge or lead acetate to improve poor wine by correcting the harsh acid taste, general interest in this subject was manifested early In Germany such disastrous results followed this adulteration of wine that decrees were issued in 1498 and 1577 which invoked the death penalty for adding lead to wine Since then the ever widening interest in lead poisoning has resulted in many attempts to discover an easy and accurate method for the detection of lead.

One of the first of these was to add sulphuric acid to wine and to ascertain the presence of lead by the formation of lead sulphate—a white precipitate In 1707 Zoller advocated the use of an extract of orpiment and lime water, which contained soluble sulphides and therefore denoted the presence of lead by the formation of a black precipitate Later, in 1784, Bergman (33a) mentioned marine (hydrochloric) and vitriolic (sulphuric) acids and sal sodae (sodium carbonate) as precipitants for lead in the analysis of water, and stated that "a brown or black sediment indicates the presence of sulphur in a state of solution," if lead is added Fourcroy and Hahnemann at about the same time suggested that acidified water saturated with hydrogen sulphide gas might be a suitable reagent Thus, it is to lead that hydrogen sulphide gas owes its place in analytical chemistry Gradually, as quantitative methods developed and chemical analysis became more exact, many other tests for lead were found A comprehensive review of these was published by Rüdisule in 1914 (392a)

Since in the examination of organic material only very small amounts of lead are involved, precautions, which are not necessary in dealing with larger quantities of lead, must be employed Definite knowledge of the conditions under which a lead salt is precipitated is a prime requisite, for the formation of plumbites in the presence of excess sodium or potassium hydroxide renders soluble a number of lead compounds which are insoluble in water alone Ammonium hydroxide precipitates lead hydroxide, but when present in excess does not dissolve the lead salt Lead sulphide is insoluble in neutral and alkaline solution and is only slightly soluble in dilute acid It is also relatively insoluble in hydrochloric acid, and the degree of acidity must be carefully adjusted when small amounts of lead are involved, if the lead is to be reprecipitated as sulphide, particularly in the presence of calcium chloride In alkaline solution most lead salts are converted to basic salts, some of which are of undetermined composition Table 1 is a list of the most insoluble of the lead salts During fine manipulations it must be remembered that filter paper adsorbs lead salts readily when they have a neutral or slightly alkaline reaction Still another possible source of error is to be found in the fact that at very high temperatures lead salts may volatilize (lead chloride boils at 900°C) and thus be lost from the substance analyzed, as for example, in ashing organic material prior to chemical examination

In other ways, too, the presence of organic material in substances to be analyzed for small amounts of lead adds complications, for many organic substances mask partially if not completely the ordinary reactions employed in tests for lead For example, lead precipitates readily from aqueous solution as the chromate, but when serum is present the addition of chromate produces no visible change Similarly, hydrogen sulphide produces a black precipitate in aqueous solutions of lead, while in serum containing the same quantity of lead it merely causes the appearance of a wine red color To avoid such errors, organic matter must be completely destroyed This is a delicate operation and if losses are to be prevented, manipulation must be extremely careful. With the proper technique, however, exceedingly small amounts of lead—1 part in 10,000,000 in urine, for instance—may be accurately detected As investigations of the toxicity of lead have progressed, methods of analysis of material containing small amounts of lead have been refined until it has become evident that the methods which are exact for inorganic compounds are inaccurate when applied to organic substances

The colorimetric sulphide method, which Pelouze (355a) first introduced in 1842, for detecting minute quantities of lead has long been a favorite It is based upon the fact that since lead sulphide is very insoluble, the degree of color in a solution of soluble sulphides should be an index of the amount of lead present As various interfering features have been discovered from time to time this method has been changed, for its great sensitiveness has made it seem valuable It does not, however, give definite proof of the presence of lead because many other substances produce exactly the same coloration. It is interesting that even as early as 1814 Orfila (344) reported that urines uncontaminated with lead often gave a positive test On this account, and also because both the size of particles and the concentration and type of salts and acids present affect the accuracy of the test markedly, other methods of analysis have supplanted it. In these, lead sulphide is still employed, but merely as a means of separating lead from other substances.

Another method which has been widely employed for the detection of lead makes use of electrolytic phenomena When an electric current passes through a solution of lead salt in nitric acid, lead peroxide (PbO_2) separates from solution at the anode This provides an excellent method of analysis for such relatively large quantities of lead as are present in ores

TABLE 1

Solubility of lead and its compounds

SUBSTANCE	SOLUBILITY	REFERENCES
	grams per liter	
Lead	1.2×10^{-2}*	Marais, H. Compt. rend. Acad. d. Sc. (1873) lxxvii, 1529
Lead bromide	8.342	Böttger, W. Ztschr. f. phys. Chem., 1903, xlvi, 521
Lead carbonate	1.75×10^{-3}	Böttger, W. Ztschr. f. phys. Chem., 1903, xlvi, 521
Lead carbonate (basic)	1.3×10^{-3}	Seidell, A. Solubilities of Inorganic and Organic Substances, N. Y., 1919
Lead chloride	10.708	Harkins, W. D. J. Am. Chem. Soc., 1911, xxxiii, 1807
Lead chromate	6.0×10^{-5}	v. Hevesy and Rona. Ztschr. phys. Chem. (1915), lxxxix, 303
Lead hydroxide	1.385×10^{-1}	Sehnal, J. Compt. rend. Acad. d. Sc., 1909, cxlviii, 1394
Lead iodide	4.70×10^{-1}	Böttger, W. Ztschr. f. phys. Chem., 1903, xlvi, 521
Lead oxide PbO	6.87×10^{-2} 18°	Pleissner, M. Arb. a. d. k. Gsndhtsamte., 1907, xxvi, 402
Lead phosphate $PbHPO_4$	1.29×10^{-2}	Fairhall, L. T. J. Indust. Hyg., 1921, vi, 160. Lead Studies X
Lead phosphate $Pb_3(PO_4)_2$	1.35×10^{-4}	Böttger, W. Ztschr. f. phys. Chem., 1903, xlvi, 521
Lead sulphate	8.24×10^{-2} 20°	Sehnal, J. Compt. rend. Acad. d. Sc., 1909, cxlviii, 1394
Lead sulphide	8.6×10^{-4}	Weigel. 1907. Quoted by Seidell, p. 365

* In water saturated with CO_2

or alloys, but unless scrupulous care is employed in the analysis of biological material, the method may lead to inaccurate results. Meillère (293) examined with this method the tissues and excreta of individuals poisoned by lead Because he was fully cognizant both of the difficulties and errors involved and of the extent to which these might influence the results, his work admits of no criticism.

Denis and Minot (94) have devised a more exact electrolytic method. In this, lead is first separated as the sulphide from a solution made alkaline with ammonium sulphide This lead precipitate is then washed with N/10 hydrochloric acid, the residue dissolved in nitric acid, and finally electrolyzed. The lead peroxide deposited at the anode (in this case a platinum dish) is next washed and treated with potassium iodide in acid solution Since iodine is quantitatively liberated under these conditions, titration with sodium thiosulphate solution indicates the quantity of lead present. Minot (306) has very recently subjected this test to severe criticism Washing the precipitate of sulphide with hydrochloric acid causes measurable loss of lead. Manganese in the substances analyzed may render results ambiguous by migrating to the anode as manganese dioxide and setting free iodine from the potassium iodide solution in acid medium. For instance, in the feces of a painter suspected of having lead poisoning, the quantity of manganese deposited in this way corresponded to 1 37 mgm. of lead; while the filtrate containing the acid washings was found by the chromate method to contain 0 29 mgm of lead Thus, unless extreme care is exercised at every stage in this test, conclusions may be very erroneous. Furthermore, the difficulties of manipulation necessitate great technical skill if minute quantities of lead are to be accurately measured by this method.

Detailed discussion of the various other methods of determining minute amounts of lead in organic material is needless After careful investigation the chromate method has proved to be the most reliable, for the processes involved purify the lead to some extent and final precipitation occurs under conditions which allow insoluble salts of no other metal to form

The chromate method. *Ashing* Before analysis tissues and feces must be freed from water by baking This may be done very rapidly by heating the material in porcelain dishes (Coors) on a hot plate until it starts to char. It should then be transferred to an electric or gas muffle furnace and ashed at dull red heat. Fecal matter usually ashes readily but the tissues form a

residue which must be repeatedly extracted before the entire char is consumed Usually most material requires re-ashing as a certain quantity of inorganic salts becomes fused and prevents complete oxidation After the first ashing the material should be cooled and extracted with dilute hydrochloric acid and hot water It is essential that at this stage *all* the ash be dissolved, for frequently lead phosphate is present as an insoluble residue that might be mistaken for silica If this residue is insoluble in hydrochloric acid it should be treated with a mixture of hydrochloric and tartaric acids (which dissolve lead phosphate), until the ash is quantitatively dissolved

Precipitation as chromate The strongly acid solution should be neutralized with NaOH HCl should then be added until it is just acid to methyl orange The next step is to saturate the cold solution with hydrogen sulphide If sulphides precipitate to any great extent during this process they may be filtered at once, but if no precipitate appears the solution, saturated with hydrogen sulphide, should be allowed to stand over night before filtration Immediately after filtration the precipitate should be washed, as lead sulphide oxidizes rapidly when in contact with air Solution of the washed precipitate in nitric acid, boiling to expel hydrogen sulphide, cooling, and finally neutralization with sodium hydroxide as indicated by phenolphthalein are then necessary After re-acidification with acetic acid, two or three drops of a saturated solution of potassium chromate should be added to the resulting solution If the solution is held against a dark background during this process a slight turbidity may be observed around the drop of added chromate in the presence of even very minute quantities of lead To hasten the reaction the solution should next be boiled for a few minutes If no turbidity is apparent, the solution should then stand over night before filtration to allow separation of the extremely small amounts of lead chromate which it may contain After filtering, all trace of soluble chromate should be washed from the filter paper, the chromate dissolved in a little dilute hydrochloric acid, an excess of potassium iodide solution added at once, and the free iodine titrated with 0 005 N sodium thiosulphate solution, a drop or two of starch being added near the end point as indicator

Preparation and preservation of standard thiosulphate solution If the standard dilute solution of sodium thiosulphate is to remain stable for any length of time it must be kept under special conditions and must be prepared with special care All distilled water should be freshly distilled and collected while still hot Glassware must be chemically clean before coming into contact with the solution In the large (20 liter) bottle which has been found most satisfactory for storing the solution, about 25 grams

sodium thiosulphate should be thoroughly mixed with 18 liters of distilled water. The bottle should then be connected with the apparatus sketched in figure 1 to prevent decomposition of the thiosulphate The two bottles, *D, D*, contain thiosulphate solution of the same strength as the

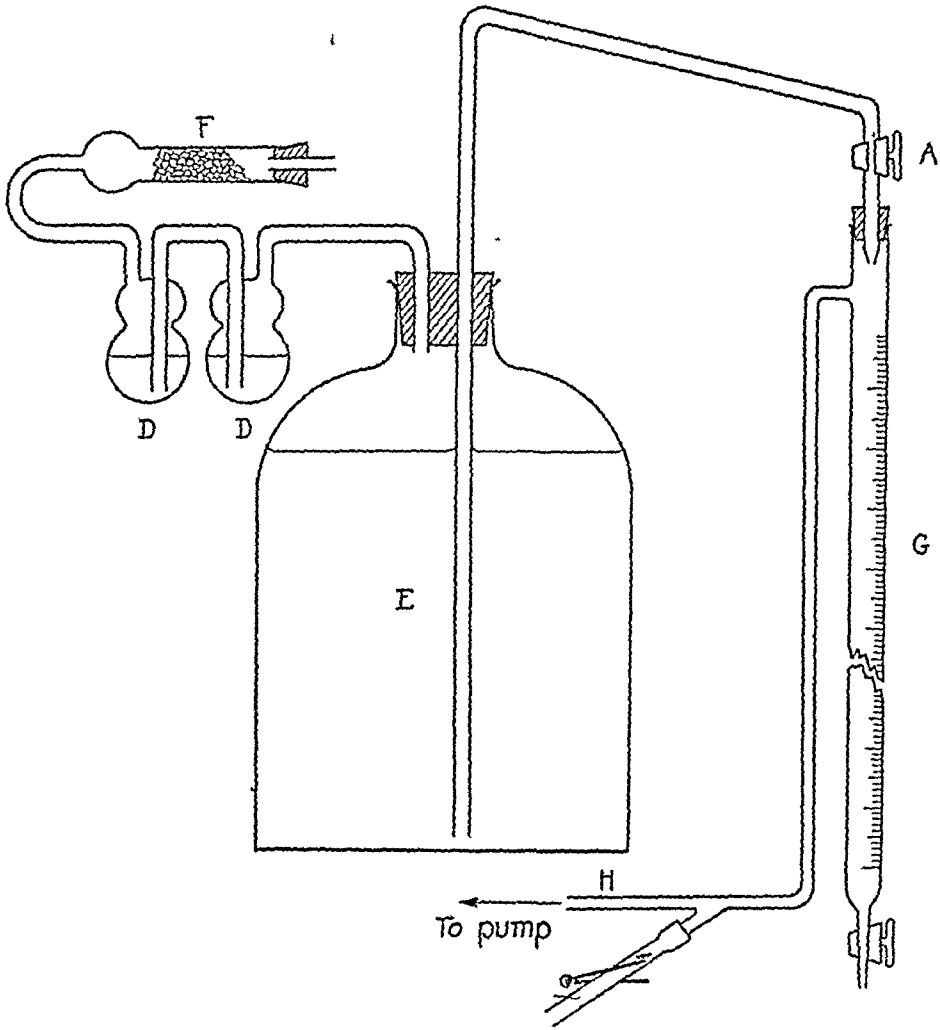

FIG 1 APPARATUS FOR STANDARD THIOSULPHATE SOLUTION

stock solution, and are arranged to allow expansion and contraction of the air in bottle *E* without forcing the wash liquid into the soda lime tube *F* The purpose of *F* is to remove carbon dioxide If the cock at *A* is closed except when the burette *G* is being filled, no air can work its way back into the bottle *E* With *H* connected to a suction pump the burette *G* is readily

filled by depressing key *B* (which fits over a piece of rubber tubing) and opening cock *A* With this arrangement the burette may be filled rapidly and air can reach the stock solution only through *F* In this way a solution of sodium thiosulphate (with a factor of 0 00558) has been kept for six months with its factor unchanged If prepared with ordinary distilled water, however, and kept without protection from CO_2 the solution changes markedly within a few days The titer of the thiosulphate solution should be determined twenty-four hours after preparing, and once a week thereafter in order to ascertain whether the solution is stable or undergoing decomposition One cubic centimeter of 0 005 normal sodium thiosulphate solution is equivalent to 0 3451 mgm of metallic lead

Sodium thiosulphate can be standardized conveniently against (approximately) 0 005 N iodine which has been accurately standardized against 0 01 N arsenious acid (465) The arsenic tri-oxide used in preparing the arsenious acid should be purified by Chapin's method (75) because the arsenic tri oxide designated "C P " or "Analyzed" frequently contains large amounts of antimony tri-oxide

Analysis of urine In analyzing urine for lead, the procedure heretofore has been to evaporate to dryness, char and ash the residue Since urine residues are particularly difficult to ash because of the large quantity of inorganic salts present, repeated extraction and ashing is necessary if all the salts are to be dissolved This makes the process burdensome The following new method in which evaporation is avoided by precipitating lead directly from urine has therefore been devised (130)

Ammonium hydroxide is added to urine until it is strongly ammoniacal This mixture is allowed to stand from one to twenty-four hours In this reaction the earthy phosphates are precipitated and lead phosphate is carried down quantitatively by *entrainment* The gelatinous mass of phosphates settles into a compact mass from which the clear lead-free liquor may be decanted and the remainder rapidly filtered by suction on a Büchner funnel The filter paper containing the precipitate ashes rapidly in a few minutes and the quantity of lead may then be determined by the chromate method outlined above

The microchemical detection of lead Very frequently the amount of lead is so minute (a few one-hundredths of a milligram) that the possibility of quantitative estimation is questionable, and under

these conditions merely the presence or absence of lead can be determined A rapid qualitative test is therefore necessary Very minute amounts of lead may be separated and detected microchemically (127) as the hexa-nitrite of potassium, lead and copper—$K_2CuPb(NO_2)_6$—a salt which crystallizes in black cubes or appears as reddish plates when seen in thin sections (figs 2 and 3)

In this test the necessary reagents are Sodium acetate 4 per cent, copper acetate 2 per cent, and acetic acid 10 per cent The capillary pipette for measuring may be prepared by drawing out a piece of 4 mm glass tubing

FIG 2 FIG 3

FIG 2 SHOWING LARGE REGULAR RECTANGULAR PLATES OR CUBES OF HEXANITRITE CRYSTALS × 85

FIG 3 SHOWING SMALL REGULAR RECTANGULAR PLATES OR CUBES OF HEXANITRITE CRYSTALS × 350

to a capillary, blowing a bulb at one end, and calibrating the stem roughly by marking two points to represent a volume of 5 cmm (about 1/10 of a normal drop) Filtering tubes may be made from glass capillary tubes with a tiny plug of absorbent cotton at one end In order to precipitate the lead, the ash from any material should be dissolved in hydrochloric acid, neutralized and re-acidified with hydrochloric acid until just acid to methyl orange One cc of a saturated solution of ammonium sulphate and one drop of the 2 per cent copper acetate solution should then be added to the material in a centrifuge tube, placed in a beaker of ice and saturated with hydrogen sulphide gas The total volume of the solution should be about 15 cc After precipitation, the sulphides should be separated by centrifu-

galization and washed at least three times by decantation After each washing the water must be completely drained from the sulphide precipitate by a capillary tube The success of this test depends a great deal upon the thoroughness with which the soluble inorganic salts are removed by washing, as they interfere very much with the test The next step is to drain the centrifuge tube containing the sulphides free from water, place it in a beaker of boiling water, and add two drops of nitric acid Some of the solution thus obtained may then be drawn up into a capillary tube from which a drop or so is placed on a microscope slide This should be evaporated to dryness Five to 10 cmm of 4 per cent sodium acetate solution should then be added and the residue completely dissolved Occasionally when bismuth is present as a result of medication with bismuth salts, a

FIG 4 SHOWING THE HEXANITRITE CRYSTALS FORMED IN THE PRESENCE OF A SMALL AMOUNT OF BISMUTH × 350

whitish precipitate will form at this point If this occurs, the acid nitrate solution in the centrifuge tubes should be evaporated to dryness and several drops of distilled water added This precipitates the bismuth as bismuth oxy nitrate which may be removed by dropping the glass capillary filtering tube into the mixture The clear solution rising through the cotton plug contains a small amount of bismuth but not enough to interfere with the test for lead (fig 4) The material should next be gathered together into one droplet and again evaporated to dryness on a slide If this is carefully done the residue consists of a glazed rim about 4 mm in diameter in which the greater part of the salts are concentrated The slide should next be chilled on ice and 5 cmm of a solution of 10 per cent acetic acid and a small crystal of potassium nitrite added This acid and nitrite crystal should be

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FIG 3 SHOWING SMALL REGULAR RECTANGULAR PLATES OR CUBES OF HEXANITRITE CRYSTALS × 350

to a capillary, blowing a bulb at one end, and calibrating the stem roughly by marking two points to represent a volume of 5 cmm (about 1/10 of a normal drop) Filtering tubes may be made from glass capillary tubes with a tiny plug of absorbent cotton at one end In order to precipitate the lead, the ash from any material should be dissolved in hydrochloric acid, neutralized and re-acidified with hydrochloric acid until just acid to methyl orange One cc of a saturated solution of ammonium sulphate and one drop of the 2 per cent copper acetate solution should then be added to the material in a centrifuge tube, placed in a beaker of ice and saturated with hydrogen sulphide gas The total volume of the solution should be about 15 cc After precipitation, the sulphides should be separated by centrifu-

galization and washed at least three times by decantation After each washing the water must be completely drained from the sulphide precipitate by a capillary tube The success of this test depends a great deal upon the thoroughness with which the soluble inorganic salts are removed by washing, as they interfere very much with the test The next step is to drain the centrifuge tube containing the sulphides free from water, place it in a beaker of boiling water, and add two drops of nitric acid Some of the solution thus obtained may then be drawn up into a capillary tube from which a drop or so is placed on a microscope slide This should be evaporated to dryness Five to 10 cmm of 4 per cent sodium acetate solution should then be added and the residue completely dissolved Occasionally when bismuth is present as a result of medication with bismuth salts, a

FIG 4 SHOWING THE HEXANITRITE CRYSTALS FORMED IN THE PRESENCE OF A SMALL AMOUNT OF BISMUTH × 350

whitish precipitate will form at this point If this occurs, the acid nitrate solution in the centrifuge tubes should be evaporated to dryness and several drops of distilled water added This precipitates the bismuth as bismuth oxy nitrate which may be removed by dropping the glass capillary filtering tube into the mixture The clear solution rising through the cotton plug contains a small amount of bismuth but not enough to interfere with the test for lead (fig 4) The material should next be gathered together into one droplet and again evaporated to dryness on a slide If this is carefully done the residue consists of a glazed rim about 4 mm in diameter in which the greater part of the salts are concentrated The slide should next be chilled on ice and 5 cmm of a solution of 10 per cent acetic acid and a small crystal of potassium nitrite added This acid and nitrite crystal should be

these conditions merely the presence or absence of lead can be determined A rapid qualitative test is therefore necessary Very minute amounts of lead may be separated and detected microchemically (127) as the hexa-nitrite of potassium, lead and copper—$K_2CuPb(NO_2)_6$—a salt which crystallizes in black cubes or appears as reddish plates when seen in thin sections (figs 2 and 3)

In this test the necessary reagents are Sodium acetate 4 per cent, copper acetate 2 per cent, and acetic acid 10 per cent The capillary pipette for measuring may be prepared by drawing out a piece of 4 mm glass tubing

FIG 2 FIG 3

FIG 2 SHOWING LARGE REGULAR RECTANGULAR PLATES OR CUBES OF HEXANITRITE CRYSTALS × 85

FIG 3 SHOWING SMALL REGULAR RECTANGULAR PLATES OR CUBES OF HEXANITRITE CRYSTALS × 350

to a capillary, blowing a bulb at one end, and calibrating the stem roughly by marking two points to represent a volume of 5 cmm (about 1/10 of a normal drop) Filtering tubes may be made from glass capillary tubes with a tiny plug of absorbent cotton at one end In order to precipitate the lead, the ash from any material should be dissolved in hydrochloric acid, neutralized and re-acidified with hydrochloric acid until just acid to methyl orange One cc of a saturated solution of ammonium sulphate and one drop of the 2 per cent copper acetate solution should then be added to the material in a centrifuge tube, placed in a beaker of ice and saturated with hydrogen sulphide gas The total volume of the solution should be about 15 cc After precipitation, the sulphides should be separated by centrifu-

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FIG 4 SHOWING THE HEXANITRITE CRYSTALS FORMED IN THE PRESENCE OF A SMALL AMOUNT OF BISMUTH × 350

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centrally placed on the dry residue and allowed to diffuse to the outer edge Such slow diffusion permits the hexa-nitrite crystals to form at various places under optimum conditions of concentration As the reagents, particularly potassium nitrite, often contain noteworthy amounts of lead, it is important to establish their purity either by re-crystallization or by the elimination of lead as sulphide wherever possible

Summary. Of the various methods of determining minute amounts of lead in biological material, the chromate has been found to be the most accurate and satisfactory For the mere detection of lead, a modified microchemical method has been developed. The analysis of urine for lead is greatly facilitated by a preliminary *entrainment* precipitation of lead phosphate with phosphates of the alkaline earths

III PHYSICO-CHEMICAL BEHAVIOR OF LEAD COMPOUNDS

While lead is both tetra-valent and di-valent, probably only the di-valent compounds are of physiological interest, with the one exception of lead tetra-ethyl which has attained some commercial importance The transformations of lead salts within the body are probably few and consist largely in the conversion of insoluble salts into a soluble form and in the reconversion of absorbed lead into an insoluble salt for storage Certain factors, such as degree of acidity and length of time of contact with the body fluids, govern the amount of lead that may enter the system after the ingestion of insoluble lead salts Thus lead oxide can enter the system more readily than lead sulphate or lead sulphide because of its greater solubility in the digestive juices

The lead compounds which most commonly enter the gastro-intestinal tract are the oxides, the sulphide, carbonate, and chromate

Carlson and Woelfel (69), who determined the solubility of some of these compounds in gastric juice found that lead carbonate is slightly more than twice as soluble as lead sulphate In their experimental work with animals lead carbonate proved much more toxic than lead sulphate, but both salts eventually caused acute symptoms of lead poisoning when administered in doses of 0 1 gram per kilo of body weight per day Lead sulphide, both natural and synthetic, although less soluble than either of these salts, is,

nevertheless, absorbed in sufficient quantities to be dangerous In a given amount of gastric juice the solubility of the carbonate is 46 per cent, that of the sulphate 9 5 per cent, and that of the natural sulphide 2.5 per cent

Lead chromate, the pigment commercially known as chrome yellow, Paris yellow, or Leipzig yellow, which is employed for calico printing or dyeing, is generally considered one of the most toxic of the lead salts It dissolves readily in dilute hydrochloric acid and consequently in the gastric juice Beck and Stegmüller (29) have observed that while in dilute acid the solubility of this salt is almost proportional to the hydrogen ion concentration, in concentrated solution the quantity dissolved is proportional to the square of the hydrogen ion concentration This is because in the latter case dichromate ions are formed

$$Pb^{++} + CrO_4^{--} + 2H^+ + 2Cl^- \rightleftarrows Pb^{++} + 2Cl^- + H^+ + HCrO_4^- \quad (1)$$

$$2HCrO_4^- \rightleftarrows Cr_2O_7^{--} + H_2O \quad (2)$$

The second of these reactions so shifts the equilibrium that more and more lead is dissolved The following equilibrium constants make this even more apparent by numerically indicating the interrelationship of the various types of reaction and the direction in which the changes will occur

$$(1) \quad \frac{(H^+)\,(CrO_4^{--})}{(HCrO_4^-)} = 3\ 7 \times 10^{-7} \qquad (3) \quad \frac{(H^+)\,(Cr\ O_7^{--})}{(HCr\ O\ ^-)} = 1\ 0 \times 10^{-2}$$

$$(2) \quad \frac{(H^+)^2\,(CrO_4^{--})^2}{(Cr_2O_7^{--})} = 3\ 4 \times 10^{-13} \qquad (4) \quad \frac{(HCrO_4^-)^2}{(Cr\ O\ ^{--})} = 2.5$$

Auerbach and Pick (22) further found that in the presence of excess sodium hydrogen carbonate, even the very insoluble salt, lead chromate, is converted into the carbonate, as illustrated in the following equation

$$PbCrO_4 + 2NaHCO_3 \rightleftarrows PbCO_3 + Na_2CrO_4 + H_2O + CO_2$$

In order to determine what possible solvent action pancreatic juice might have upon these compounds, the action of sodium carbonate and sodium hydrogen carbonate upon the carbonate and sulphate of lead has been carefully studied by Auerbach and Pick (21)

When the total sodium concentration is below 0 077 N the reaction with sodium carbonate is

$$3PbCO_3 + Na_2CO_3 + 2H\ O \rightleftarrows 2PbCO_3 \cdot Pb\ (OH)_2 + 2NaHCO_3$$

Above this point, however, the double salt, basic sodium lead carbonate ($NaPb_2(CO_3)_2OH$), is formed. Lead sulphate reacts with sodium hydrogen carbonate according to the equation

$$PbSO_4 + 2NaHCO_3 \rightleftarrows PbCO_3 + Na_2SO_4 + CO_2 + H_2O$$

The reaction is reversible and therefore depends upon the CO_2 concentration It is possible that the reaction with sodium hydrogen carbonate may occur to some extent in the lungs where the particles are bathed in a medium containing small amounts of this salt, and thus account for the fact that lead chromate, although very insoluble, enters the circulation readily from the respiratory tract It is evident therefore that very insoluble lead salts may be slowly converted into lead carbonate when in contact with pancreatic

TABLE 2

The solubility of various lead compounds in blood serum

NUMBER	SUBSTANCE	SOLUBILITY IN SERUM AT 25°C	SOLUBILITY IN H_2O
		grams per liter	*grams per liter*
1	$PbCO_3$	0 0333	0 0017 18° (1)
2	$PbSO_4$	0 0437	0 044 24,95° (2)
3	$PbCrO_4$		0 00001 25° (3)
4	PbO	1 1520	0 0171 20° (1)
5	Pb	0 578	

(1) Pleissner, M Chem Centr -Bl , 1907, ii, 1056
(2) Bottger, W · Ztschr f phys Chem , 1903, xlvi, 604
(3) (von) Hevesy, C Ztschr f anorg Chem , 1913, lxxxii, 328

juice or with such other body fluids as blood plasma While lead carbonate itself is somewhat insoluble, it is far more soluble than many lead compounds from which it can thus be formed, and the chances of its entering the circulation are therefore relatively great

While the entrance of lead or its compounds into the gastro-intestinal tract is an important factor in the etiology of plumbism, absorption from the lungs produces lead poisoning much more directly (see section V) In industry, workmen are commonly exposed to the dust of white lead, of lead oxides, or of sandpapered paint Fine particles of these various compounds are inhaled and very soon after becoming lodged in the alveoli may produce severe lead poisoning. Phagocytosis (137) and direct absorption of dissolved lead compounds, as well as gradual diffusion from slowly dissolving particles in contact

[illegible] epithelial cells of the alveoli must play some role in this [illegible]. While metallic lead and lead oxide are strikingly soluble in [illegible] and lead carbonate is more soluble than in pure [illegible] the solubility of lead carbonate in serum saturated with CO_2 [illegible] as that in [illegible] solution as determined by [illegible].

The marked solubility of lead oxide and metallic lead in serum is significant, for in the lead industry poisoning develops rapidly after exposure to lead fume and to lead oxide dust. Legge and Goadby (252) point out that the incidence of lead encephalopathy is higher among people who manufacture lead oxides than in any other lead industry. Therefore, the chemical transformations that lead salts may undergo in the body assume an important and very practical aspect.

TABLE [illegible]

Effect of CO_2 on the solubility of lead compounds in serum

[illegible]	[illegible]	[illegible]
		[illegible]
1	$PbCO_3$	0.[illegible]25
2	$PbSO_4$	0.0300
3	PbO	[illegible]1500
4	Pb	0.[illegible]

Since lead is slowly accumulated in the body and deposited in the bone tissue, probably as a phosphate (see page 25) some knowledge of the reactions and equilibria involved in the formation of the phosphates of lead is necessary. The conditions under which these phosphates form at room temperature are not very well known and most investigations have been carried out only under synthetic rather than equilibrium conditions

Investigation (125) has demonstrated that under equilibrium conditions di-sodium phosphate and lead chloride react to form di-lead phosphate, $PbHPO_4$. This occurs only under equilibrium conditions, for when the two reacting substances are first mixed, tri-lead phosphate is formed. The curve (fig 5) illustrating the changes in hydrogen ion concentration with increasing amounts of sodium phosphate shows that the acidity rises to

maximum and then decreases. If the two substances react, as is generally stated, according to the equation,

(a) $$PbCl_2 + Na_2HPO_4 \rightleftarrows PbHPO_4 + 2NaCl$$

the solution should remain neutral, whereas if tri-lead phosphate is formed,

(b) $$3PbCl_2 + 2Na_2HPO_4 \rightleftarrows Pb_3(PO_4)_2 + 4NaCl + 2HCl$$

it should become acid

In figure 5 it may be seen that the solution becomes more acid with each successive addition of di-sodium phosphate until there is present nearly

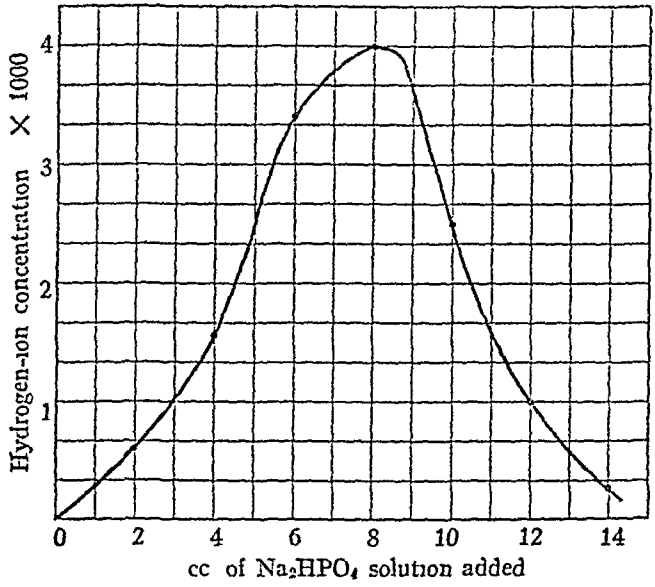


FIG 5

the amount of phosphate theoretically necessary to precipitate the lead as tri-lead phosphate Although under these conditions the amount of sodium phosphate is only 66 per cent of that required to precipitate the lead as di-lead phosphate, the filtrate is lead-free From this point on, the acidity decreases because of the excess sodium phosphate Thus it is evident that under *synthetic* conditions or in slightly alkaline solution the tri-lead is formed, while under *equilibrium* conditions di-lead phosphate is formed

The transformation of di-lead phosphate into the tri-lead salt takes place in the following manner.

$$3PbHPO_4 \rightleftarrows Pb_3(PO_4)_2 + H_3PO_4$$

when the salt is suspended in boiling water A constant increase in the

acidity of the solution, as well as the change in microscopic appearance of the salt, is evidence of this reaction. This change is very slow and requires several hours even under the most favorable conditions

The phase diagram in figure 6 shows the range of existence of lead phosphates over a wide range of acidity In the area *ACE* solution is complete and no solid phase is present Experiments 1 to 10 inclusive are plotted along the straight line *AC* The lines connecting these two points with

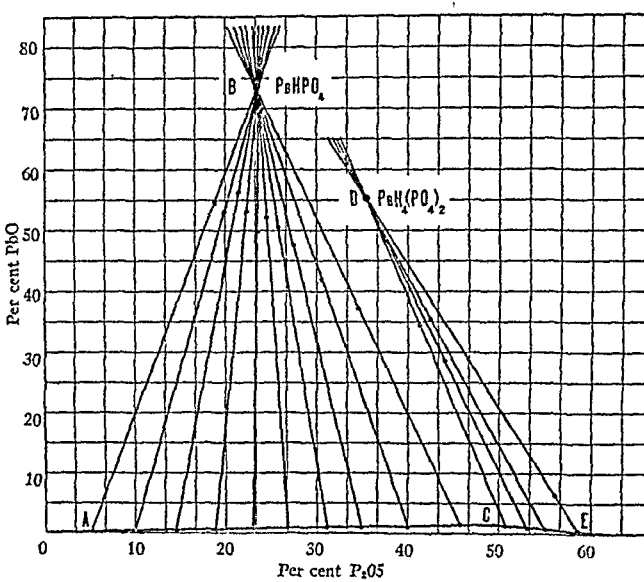


FIG 6

those representing the composition of the solid residues, converge at *B* which represents the compound, di-lead phosphate As these experiments include a wide range of concentration, this point is quite satisfactorily established The area *ABC* therefore represents a mixture of the solid phase with various solutions At *C* there is a maximum amount of lead in the solution phase This point, the intersection of the two branches *AC* and *CE*, falls where a constant solution would exist in contact with the two phases di-lead hydrogen phosphate and mono lead tetra-hydrogen

phosphate The branch *CE* (experiments 11 to 14) illustrating the solid phase $PbH_4(PO_4)_2$ in contact with varying solutions, and the lines representing the compositions of the solution phase $PbH_4(PO_4)_2$ in contact with varying solutions, and the lines representing the compositions of the solution phase and solid phase very nearly coincide with point *D*, representing the theoretical composition of $PbH_4(PO_4)_2$ when they are projected

Thus the phase diagram indicates quite clearly the stability of the di- and mono-lead phosphates at various acid concentrations and furnishes means of predicting the result of bringing various mixtures to equilibrium. Any mixture represented by points within the area *ACE* for example must yield a solution with no solid phase The composition of any compound formed by the empirical mixture of substances falling within the limits of the area *ABC* may be similarly foretold The short branch *CE* illustrates the solubility of mono-lead tetra-hydrogen phosphate in phosphoric acid at 25°C. and shows that this rapidly decreases as the concentration of acid increases

Since tri-lead ortho-phosphate cannot exist at equilibrium in even slightly acid media, the behavior of lead within the body is the more easily explained While lead phosphate under the normal conditions of alkalinity remains inert and harmless, any *local* production of acid must transform the tri-lead salt to di-lead phosphate Since the solubility of the latter salt is 12 9 mgm per liter and that of the tri-lead is only 0 13 mgm per liter, the solubility of lead is thus increased an hundred-fold

This shows, therefore, that the two lead phosphates of physiological interest are tri-lead and di-lead hydrogen phosphate The transformation of one into the other is governed by conditions within the body and may have an important bearing upon the health of the individual. Indeed, this may account for the fact that lead may be stored in the body for years without causing any ill effects until some such influence as deficient diet or acute infection alters the reaction of the tissues and increases the amount of lead in circulation

Summary. It is apparent from the foregoing discussion that the products of the reaction between secondary sodium phosphate and a lead salt are tertiary lead phosphate and acid This lead salt is very stable at the normal hydrogen ion concentration of the body, but is

sensitive to slight changes in acidity and passes into the more soluble di-lead salt readily The marked solubility of lead and lead oxide in such body fluids as blood plasma may account for the high incidence of lead poisoning among workers exposed to lead fume and lead oxide dust Both gastric and pancreatic juice may transform insoluble lead salts into more soluble forms and may therefore cause an increased absorption of lead compounds

IV THE TRANSPORTATION AND DEPOSITION OF LEAD IN THE BODY

Transportation of lead in the body Definite investigation of the form in which lead is transported in the blood stream is very difficult because during plumbism the amount present in the blood at any given time is so minute that isolation and identification are almost impossible Certain considerations, however, suggest that whatever lead is present exists as the phosphate,—probably in colloidal form, rather than as the albuminate as Sir Thomas Oliver suggests (337) The chief of these is the fact that in plasma the proportion of lead to inorganic phosphate is very small Because lead phosphate is extremely insoluble, any lead should therefore combine with the phosphate Experiments (page 34) also indicate that lead is stored in the body as phosphate, while electrometric measurements (*vide infra*) in which lead salts were added to serum indicate that the lead is in combination with the inorganic phosphate group

Although the phosphate is thus formed it does not precipitate from the blood serum when present in small amounts, but remains suspended as peptized or highly dispersed colloidal lead phosphate Other lead salts behave in the same way when they are formed in serum Lead chromate, for instance, apparently remains in solution The fact that lead can be transported in the blood stream as an insoluble salt is therefore not surprising When a clear solution of highly dispersed lead phosphate in serum is treated with hydrogen sulphide, part of the phosphate is changed to colloidal lead sulphide and although the solution remains perfectly transparent its color changes to a deep red This shows that the sulphide is formed in a highly dispersed condition Similar peptizing effects can be shown with substances other than serum Thus, in the presence of cane sugar lead phosphate may be so highly dispersed that the solution is

as transparent as distilled water If hydrogen sulphide gas is passed through such a preparation the change in color is similar to that already mentioned, here again there is no precipitation, for the lead sulphide remains highly dispersed and apparently in true solution. These facts lead one to believe (*a*) that lead could hardly be present in the blood stream except as lead phosphate, and (*b*) that it is carried in colloidal form (see also page 29).

Tests carried out with very refined technique have shown that only minute quantities of lead are present in the blood at any given time For instance, the blood of animals which have been fed with lead salts usually contains only a few thousandths of a milligram of lead as long as the animals are in apparent good health When such complications as acidosis develop and the animal becomes sickly, the quantity of lead in circulation increases slightly. In a case of fatal lead encephalopathy in man there were in the blood 2 8 mgm of lead per liter—an amount which corresponds to 3 64 mgm of tertiary lead phosphate. Since only 0 13 mgm of this lead salt dissolves in a liter of pure water (246) the blood in this case contained about thirty times as much lead as could be dissolved in a corresponding volume of water. This is further evidence that *in vivo* lead is transported as colloidal lead phosphate

That the greater part of the lead in the blood is held by the plasma and that but little is carried in the cells has been ascertained by experimentation In a rabbit with lead poisoning the lead in the blood amounted to 0 75 mgm of which 0.59 mgm was in the plasma and 0 16 mgm in the red cells Thus, practically 80 per cent of the lead was carried by the plasma

When a soluble salt of lead is added to blood serum, a precipitate, probably lead albuminate, an organic salt, forms at first and then dissolves This is not particularly insoluble in water and is quite sensitive to changes in acidity. The maximum precipitation occurs at pH 5 8 which is probably the iso-electric point, for above or below this the salt re-dissolves The precipitate is white and separates easily from solution When dissolved in a few drops of dilute hydrochloric acid and treated with hydrogen sulphide gas, no change in color occurs until after a short time lead sulphide precipitates rapidly.

Since some authors have assumed that lead is carried in the blood

as an albuminate, the effect of the serum proteins upon the electrical behavior of dissolved lead salts is of interest Measurements of the lead ion concentration should demonstrate whether the lead salt in solution is complex and whether the lead is in the anion If sodium hydroxide is added to a solution of lead salt the lead hydroxide which at first precipitates is redissolved because of the formation of the complex sodium plumbite

The successive steps are indicated in the following equations

a) $$Pb^{++} + 2OH^- \rightleftarrows \underset{\text{solid}}{\underline{Pb(OH)_2}}$$

b) $$Pb(OH)_2 + NaOH \rightleftarrows NaHPbO_2 + H\,O$$

c) $$NaHPbO_2 \rightleftarrows Na^+ + HPbO_2^-$$

When an excess of sodium hydroxide is used, the positively charged lead ions (Pb^{++}) are wholly used up—the lead appears in the negatively charged group $HPbO_2^-$ Since in reaction (*b*) when there is excess of sodium hydroxide present, equilibrium lies in the direction of the formation of sodium plumbite, the lead ion (Pb^{++}) concentration must be enormously reduced Electrometric measurements of the degree of this reduction may be made by determining the electromotive force of the concentration cell,

$$Pb \mid 0\,1\ M\ KCl \text{ saturated with } PbCl \mid NH_4NO_3 \text{ saturated} \mid$$
$$0\,01\ M\ KCl \text{ saturated with } PbCl \mid Pb$$

which is markedly increased by the addition of sodium hydroxide to one of the half elements of the electrical cell

Without discussing the several factors that must be taken into account in making measurements of this character, the method of determination may be briefly described as follows

Two beakers, one containing dilute, the other strong lead chloride solution, are connected by means of an inverted U tube filled with conducting salt solution If a rod of pure lead is inserted in each, a so-called concentration cell is formed which will "run down," when the cell is short-circuited by connecting the two electrodes During this process lead passes into solution in the beaker containing dilute lead chloride, acquires a positive charge, and the lead ion concentration increases In the more concentrated solu-

tion lead ions give up their positive charge and there is a tendency for lead to plate out on the electrode By means of a sensitive galvanometer introduced into the circuit, it is possible to demonstrate that the magnitude of the electromotive force depends upon the difference between the lead ion concentration in the two half cells or elements. As the lead ion concentration decreases in one of the beakers while that in the other remains constant, the electromotive force increases Therefore, if the lead ion concentration (C) in one solution is known and the electromotive force of the cell is determined, the lead ion concentration (C') of the other solution may be computed from the Nernst equation $e\,m\,f. = 0\ 058\ ln\ \frac{c}{c'}$.

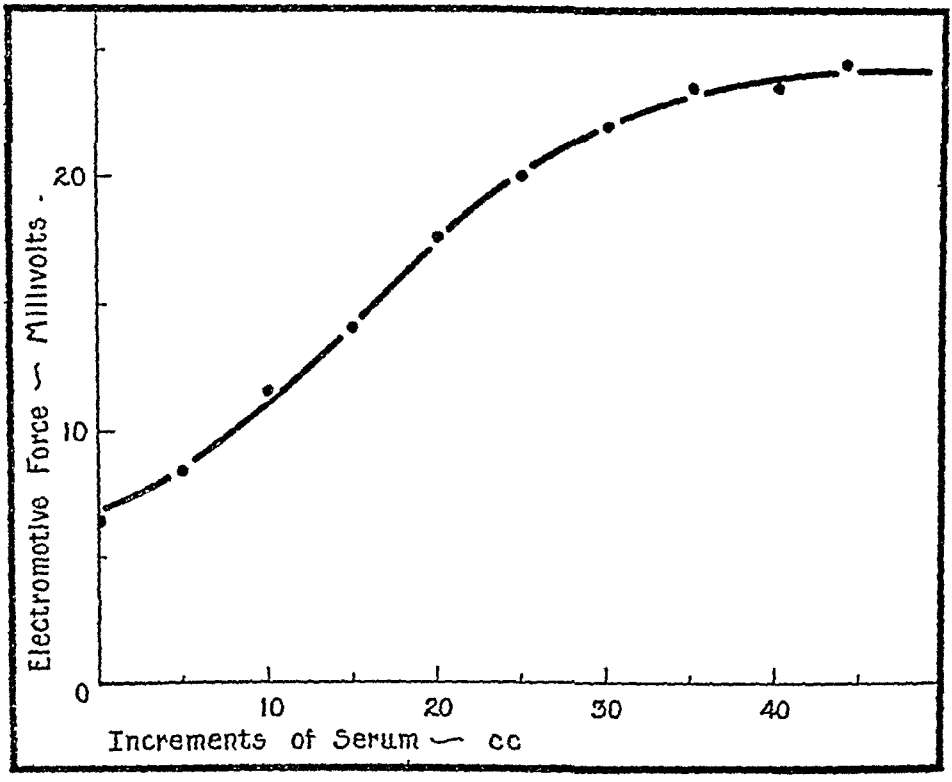


FIG 6B

A typical experiment with serum was performed as follows·

Lead chloride in amounts containing from 1 to 10 mgm. of metallic lead was added to 0 1 M KCl solution containing 2 cc of rabbit serum. These mixtures in volumetric flasks were then diluted with 0 1 M KCl to exactly 50 cc. With the various solutions thus prepared, amalgam cells were set up so that measurements of the electromotive force could be made at inter-

vals until equilibrium was apparently established Similar dilutions of lead chloride alone in 0 1 M KCl were prepared for another series of amalgam cells to provide data on the extent of electrical dissociation of lead chloride Amalgam cells containing 0 1 M KCl saturated with $PbCl_2$ were used as reference cells When compared with a standard calomel cell the values checked favorably with those obtained by Lewis (263)

The experimental results are collected in table 4 From these figures it may be seen that the first decrease in lead ion concentration becomes less marked as the ratio of lead to serum increases This is merely because at first some of the lead is thrown out of solution as a protein precipitate With comparatively large amounts of lead (above 5×10^4 mols), the precipitate re-dissolves and the

TABLE 4

Effect of small amounts of lead chloride on serum proteins

TOTAL LEAD PRESENT $\times 10^4$ MOLS	MEASURED LEAD ION CONCENTRATION $\times 10^4$ MOLS	THEORETICAL LEAD ION CONCENTRATION $\times 10^4$ MOLS	DIFFERENCE $\times 10^4$ MOLS
1	0 05	0 95	0 90
2	0 60	1 50	0 90
3	1 90	2 25	0 35
4	2 44	2 60	0 16
5	2 80	2 80	0 00
6	3 08	3 09	0 01
7	3 20	3 20	0 00
8	3 53	3 53	0 00
9	3 70	3 75	0 05
10	3 90	4 00	0 10

lead is ionized normally If the serum proteins and lead had combined to form a complex salt in which lead was present in the anion, the Pb^{++} ion concentration would have markedly decreased Therefore we can state that there is no evidence that lead is carried in the blood stream as a complex salt in which the lead is in the anion

When a small quantity of lead is titrated with a large quantity of serum, the results are different The small initial precipitate dissolves at once in the excess serum, but the lead ion concentration decreases slowly This is shown in table 4 and in figure 6B in which the increments of serum are plotted against the values for electromotive force which are inversely proportional to the lead ion con-

centration. The curve is somewhat S-shaped with its steepest inclination at the point where about 15 cc of serum was added This corresponds approximately to the point at which all the lead would be combined with the inorganic phosphates of the serum These facts justify the conclusion that the reduction in the lead ion concentration is not due to combination of lead with the proteins of the blood but to its combination with certain of the mineral constituents of serum (probably the phosphate ions because the carbonate is more soluble) when a large excess of serum is present No precipitate is visible because the lead phosphate is highly dispersed by the peptizing action of the serum proteins. All these facts, therefore, point to the conclusion that lead is transported in the blood stream as highly dispersed or colloidal lead phosphate

The deposition of lead salts. An understanding of the conditions and reactions underlying the calcium exchange in bone is quite essential in a study of the deposition of lead salts in the skeleton The early experiments of König (240) and the later work of Lehnerdt (258) and of Stoltzner (446) on the substitution of strontium for calcium demonstrate quite clearly that the calcium in bone tissue may be replaced by other elements with somewhat similar reactions Until the properties of some of its compounds are known, however, lead would hardly be considered one of these, and the remarkable deposition of lead in bone tissue (see section VI) would scarcely be anticipated Lead resembles calcium in that it forms both an insoluble carbonate and an insoluble phosphate This lead phosphate is one of the few insoluble phosphates which does not react with most chemical reagents Neither the mechanism of the deposition of lead in bone nor the process by which calcium is deposited are fully understood Although certain conditions are known to retard the deposition of calcium while others increase it, the actual process by which calcium is laid down still remains obscure Of the several theories advanced to account for calcium deposition, none is completely satisfactory

Wells (488) has discussed some of the earlier theories, among them the conception that calcium fixation or deposition is the result of chemical reaction, and the hypothesis that calcium is first deposited as a soap which

later becomes transformed into the corresponding salts by contact with the carbonates and phosphates of the blood Experimental work has neither confirmed this idea of soap formation nor demonstrated that cartilage is transformed into normal bone by passing through an intermediate soap stage, and Wells, after a careful study of this problem, has concluded that calcification is a process of adsorption of calcium by hyaline tissue which has a more or less specific affinity for calcium Other hypotheses have been advanced more recently Mellanby (295) has concluded from his study of rickets in children that the deposition of calcium in the bones is initiated by some accessory food factor His experiments on puppies demonstrate that although rickets may be produced while they are very young, beyond a certain age the disease seldom develops, apparently because of a process of self-cure The same seems to hold true for children Beyond a certain age (two years) the development of active rickets is rare, probably because of the development of some essential process or secretion Robison (389) has found that an enzyme in the ossifying cartilage of young rats and rabbits rapidly hydrolyses the hexose-mono-phosphoric acid ester present in small amounts in the plasma and in relatively large amounts in the corpuscles of the blood, and thus sets free phosphoric acid

The theory that increasing the phosphate ion concentration ($HPO_4^{=}$) of the bones in this way would result in a local precipitation of calcium phosphate, is ingenious but does not explain the simultaneous deposition of calcium carbonate which is known to form 10 to 15 per cent of the inorganic constituents of normal bone This brief review of the various theories shows how inexact is our knowledge of the mechanism of the deposition of calcium

Perhaps the most stimulating hypothesis thus far suggested is that of Wells, i e, that the deposition of calcium is to be attributed to an initial adsorption of calcium by the hyaline cartilage In 1904 Pfaundler (358), by means of discs of gelatin, made a systematic study of the physical process of absorption of various crystaloids, and concluded that gelatin has a specific ionic affinity for calcium Freudenberg and Gyorgy (148) have recently conducted an extensive investigation based to some extent on this earlier work They found that cartilage immersed in calcium chloride solution absorbed calcium in amounts which were regulated by the hydrogen ion concentration The more acid the solution the less calcium was absorbed They also found that cartilage removed magnesium, barium,

and strontium from solution in the same way. It is, however, apparent from their results that even when the time of contact was greatly prolonged absorption was not complete Such calcification as this by chemical or physical combination of calcium and tissue protein is not, of course, calcification in the histological sense, for there is no such structural differentiation as appears in true calcified tissue When metals like lead are fixed by cartilage, they penetrate in an even mass to various depths depending upon concentration, time of contact, etc After this treatment cartilage presents a picture so entirely different from that of normal calcified tissue that the dissimilarity between the two is at once evident

Histological examination of bone shows that calcium salts exist as such between the cells and not chemically bound to the protein. That the calcium is deposited in the intracellular spaces of the bone tissue as a secretion of the osteoblasts has been generally assumed by histologists (82). Quite recently Rabl (372) has been able to differentiate between calcium carbonate and calcium phosphate on the one hand and soluble calcium salts in calcifying tissues on the other For this purpose he used ammonium oxalate because it reacts only with the dissolved calcium salts and not with the carbonate or phosphate By this method the presence of dissolved calcium salts not only in the ground substance but also in the cells of growing bone tissue may be demonstrated, but without giving any clue as to the mechanisms of the absorption and deposition of calcium in the intracellular spaces, or the process by which calcium phosphate and carbonate are formed In bone formation 85 to 90 per cent of calcium phosphate as compared with 15 to 10 per cent of calcium carbonate is deposited. This ratio is invariable and must depend upon rather accurately defined equilibrium conditions These in turn are evidently regulated by the inorganic constituents of the blood plasma The occurrence of a pathological increase of calcification in certain areas having an impoverished blood supply has been mentioned frequently (331), and this phenomenon may have an important bearing on the explanation of the various processes involved

On the other hand, Tanaka has shown (452) that calcium phosphate injected in areas where the blood supply is relatively rich is absorbed with great rapidity, and (452) that if solid tri-calcium

phosphate is shaken for twelve days at body temperature in a solution containing the inorganic salts of the blood plasma (0 9 per cent NaCl, 0 1 per cent Na_2CO_3, and 0 1 per cent $NaHCO_3$) the calcium is bound—85 19 per cent as $Ca_3(PO_4)_2$ and 12 76 per cent as $CaCO_3$ This experiment *in vitro* is not conclusive because the solution was renewed daily and if continued sufficiently long all the calcium phosphate would be converted to calcium carbonate In other words, there was no equilibrium established in a chemical sense These experiments illustrate how difficult it often is to duplicate experimentally conditions or changes which occur within the body

While lead resembles calcium in many respects—a fact which accounts for its deposition in bone tissue—there are several important differences between the two, particularly with regard to the solubility of certain salts Lead phosphate is much more insoluble than lead carbonate, for instance, while calcium phosphate is more soluble than calcium carbonate Therefore the mechanism which tends to precipitate the bulk of calcium in bone as calcium phosphate—the more soluble salt—would precipitate lead most readily as lead phosphate—a very insoluble salt Again, calcium carbonate is sensitive to changes in carbon dioxide concentration, and it is not unlikely that the calcium stored in this form in the bones tends to regulate to some extent the amount of calcium in circulation The solubility of $CaCO_3$ is only 9 6 mgm per liter, whereas the solubility of $Ca(HCO_3)_2$ is 385 mgm per liter Furthermore, carbonic acid has been shown to exert a pronounced solvent action upon calcium phosphate (278), while it converts lead phosphate only very slowly to the di-lead salt Fluctuations in the concentration of CO_2 would therefore regulate and keep fairly constant the quantity of calcium in the blood stream, whereas they would not affect the deposited lead salt

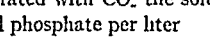
Numerous experiments have been performed (129) to determine the composition of the lead salts stored in the body Minot (307) has shown that storage occurs in the skeleton. Bones of animals experimentally poisoned with lead were therefore studied to determine the chemical composition of the deposited lead salt, for it had been previously suggested that lead is deposited in the body as an al-

buminate or carbonate. Both these salts are soluble in acetic or similar weak acids, but the repeated attempts made to dissolve lead from bone tissue with these acids have always resulted in failure It is, however, possible to remove lead from bone with nitric acid in which lead phosphate is soluble To determine the exact nature of the lead compound in bones is obviously very difficult, for the amounts of calcium phosphate and carbonate present are enormously greater than the quantity of lead deposited. Prévost and Binet (368) came to the conclusion in 1889 that lead was stored as phosphate because of its insolubility in sulphuric acid and ammonium acetate, and its solubility in nitric acid Tartaric acid, which is a good solvent for lead phosphate, removes lead from bone tissue but dilute hydrochloric acid, which would dissolve lead only slightly, must be present to keep the insoluble calcium tartrate in solution Sulphurous acid completely dissolves the calcium from bone as acid calcium sulphite, $Ca(HSO_3)_2$, but leaves the lead unaffected Therefore lead in bone tissue is in the form of a less soluble salt than the carbonate or albuminate

Because the amount of lead present is so minute—usually about 10 to 20 mgm in 150 grams of bone—it has been impossible to separate the lead compound and to identify it definitely as lead phosphate. But experiments have been performed on the different lead salts which throw some light on the problem When circulating lead comes into contact with the organic matrix of bone it is doubtless deposited much as is calcium except that it can be deposited in any quantity only as the phosphate The solubility of lead phosphate in water is 0 13 mgm per liter, while that of lead carbonate is 1 7 mgm per liter When precipitated as phosphate by di-sodium hydrogen phosphate, lead is in the form of the tri-lead salt which is transformed slowly in neutral or slightly acid solution into secondary lead phosphate ($PbHPO_4$) This di-lead salt always forms under equilibrium conditions (126) but in a slightly alkaline medium similar to that of the body, the tertiary salt is the stable form It is in this very insoluble condition that lead is probably stored in the bones. Under normal conditions it may remain inactive for comparatively long periods, but when the alkalinity decreases, as in acidosis, the equilibrium swings toward the most soluble lead salts so

that lead may be set free (see section IX) and cause acute symptoms of lead poisoning

Solubility determinations of di-lead phosphate prepared by the synthetic method (Series I) (4), and under equilibrium conditions (Series II) have been performed The results are summarized in table 5 The average solubility (0 0129 ± 0 0004 gram per liter) proved to be approximately one hundred times that of tri lead phosphate This pronounced difference is important, for any variation in local reaction, such as increased acidity, may affect to a marked degree the quantity of lead in circulation Even as weak an acid as carbonic can react slowly with tri-lead phosphate in the following manner



$$Pb_3(PO_4)_2 + H_2CO_3 \rightleftarrows PbCO_3 + 2PbHPO_4$$

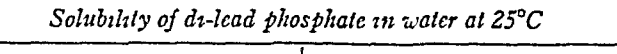
The solubility of this tri-lead salt in carbonic acid corresponds to that of the di lead salt in pure water When shaken for several hours at room temperature in water saturated with CO_2 the solution phase contained as much as 13 0 mgm of lead phosphate per liter

The failure of weak acids to dissolve lead *in vitro* from bones containing lead is undoubtedly in part dependent upon the buffer action of calcium phosphate and in part upon the decreased solubility of lead phosphate in the presence of excess phosphate ions But a factor of probably greater importance than either of these is the fact that *in vivo* the production of acid is local and proceeds from the interior of the cell outward, whereas *in vitro* the action of the acid is from the outside in

If normal and experimental conditions are considered from the viewpoint of hydrogen ion concentration, deficiencies of the experiments *in vitro* become more apparent Local production of acid within the cell must raise the hydrogen ion concentration far more than can the mere addition of the same amount of acid diffused through a large volume of solution Therefore lead in the bone must be more affected by local changes than it is by variations in the circulating medium which bathes the cells and by buffer action which maintains a nearly constant hydrogen ion concentration

When tri-lead phosphate is shaken with lactic acid the phosphate neutralizes the acid (table 6 and fig 7) Since lead lactate is soluble,

much more lead is carried into solution than when the lead salt is mixed with carbonic acid.



$$Pb_3(PO_4)_2 + 2CH_3CHOH\ COOH \rightleftarrows Pb(C_3H_5O_3)_2 + 2PbHPO_4$$

That local changes in reaction of the tissues are among the factors determining how much lead can pass into circulation, is therefore not unlikely This solubility of tri-lead phosphate in lactic acid is particularly striking and may account for the fact (see section VI)

TABLE 5

Solubility of di-lead phosphate in water at 25°C

SERIES I—SYNTHETIC $PbHPO_4$		SERIES II—EQUILIBRIUM $PbHPO_4$	
Grams per liter	Deviation from mean	Grams per liter	Deviation from mean
0 0134	0 0005	0 0132	0 0003
0 0120	0 0009	0 0120	0 0009
0 0129	0 0000	0 0143	0 0014
0 0130	0 0001	0 0124	0 0005
0 0132	0 0003	0 0125	0 0004
		0 0133	0 0004
0.0129	Average	0 0129	Average

TABLE 6

Solubility of lead phosphates in lactic acid at 25°C

NUMBER	LACTIC ACID NORMALITY	DISSOLVED DI-LEAD PHOSPHATE	DISSOLVED TRI LEAD PHOSPHATE
		gram per liter	*gram per liter*
1	0 047	0 141	0 686
2	0 093	0 197	1 175
3	0 186	0 283	1 794
4	0 465	0 458	2 296
5	0 930	0 484	2 661

that only traces of lead are found in muscle tissue, while large amounts may be present in bone

The absorption of lead by bone Experiments have shown that pieces of bone taken from an animal immediately after death absorb lead from a dilute solution of lead salt at a rate which depends upon the size of the bone particles This lead is not removed by the formation of an insoluble albuminate, for the freer the bone from protein material the more efficiently is the lead taken from solu-

tion These interesting facts have led to an investigation of the power of crushed bone to absorb lead

In a series of experiments bone was carefully freed from fat by repeated extraction with chloroform and ether, and then from protein material by extraction with boiling salt solution and water until the biuret test was no longer positive The crushed bone was carefully graded by sifting, and was shaken in the shaking machine with lead chloride solution at various concentrations Because of the mutual grinding action of the bone particles under these conditions a certain amount of bone becomes more finely divided It is, therefore, more satisfactory to allow the solution to flow continually over a bed of crushed bone The apparatus shown in figure 8 was devised for this, and since it is automatic, uniform conditions may

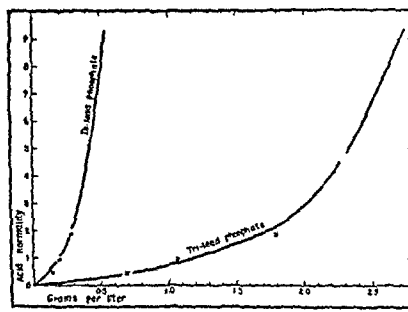


FIG 7 SOLUBILITY OF LEAD PHOSPHATES IN LACTIC ACID AT 25°C

easily be maintained That lead is quickly removed from solution under such conditions is shown by table 7 and figure 9 The rate of removal is high—at the end of fifteen minutes the lead is completely withdrawn from solution Such other substances as charcoal and cellulose which adsorb lead, or permutite which interacts with it chemically, are relatively ineffective in removing lead from solution when compared with crushed bone

To demonstrate that lead is removed from solution by replacement of the calcium in the calcium phosphate of bone, required but little experimentation Lead solutions of known concentration were pumped over crushed bone until the lead was completely absorbed. The filtrate containing the calcium was then analyzed and the amount of calcium liberated compared with the amount of lead absorbed Table 8 and figure 10 indicate that the

amount of calcium liberated increases as larger quantities of lead are absorbed. The process is, therefore, one of replacement

The degree of absorption of lead by crushed bone depends upon the hydrogen ion concentration (table 9 and fig 11) Lead is not

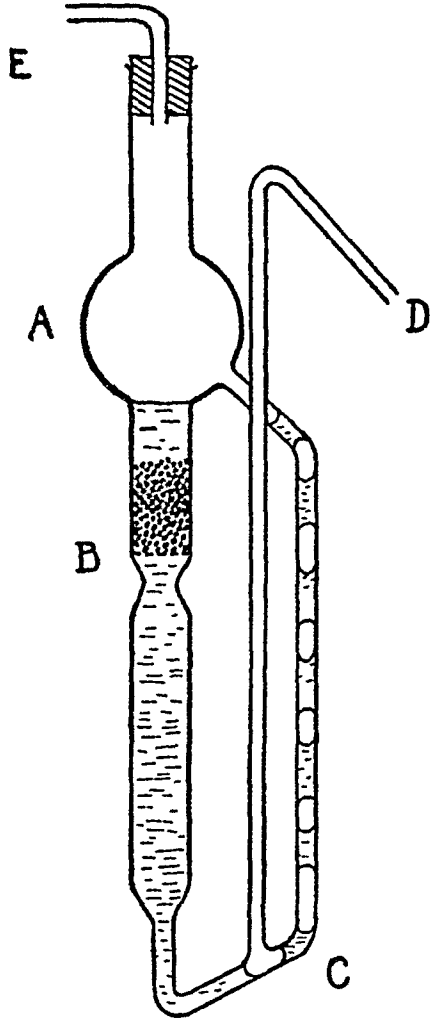


FIG 8 CIRCULATING PUMP

removed very effectively from the more acid solutions, but as the acidity falls lead absorption increases until at pH 7 8 the maximum absorption occurs Below this point lead is again absorbed in decreasing amounts It is of biological interest that the range of maximum absorption *in vitro* lies between pH 7 4 and 7 8

That lead replaces calcium in bone as phosphate rather than by uniting directly with the protein of the organic matrix has been demonstrated. When the organic material in bone particles is destroyed by ignition, lead is much more readily taken from solution than when the calcium phosphate of the bone is removed by decalcification There is a distinct difference between the removal

TABLE 7

Rate of absorption of lead

NUMBER	INITIAL AMOUNT OF LEAD IN SOLUTION	FINAL AMOUNT OF LEAD IN SOLUTION	TIME OF ABSORPTION
	mgm	*mgm*	*min*
1	25	15 89	¼
2	25	10 78	1
3	25	6 64	2
4	25	4 44	3
5	25	1 49	6
6	25	0 30	9
7	25	0 12	12
8	25	0 00	15
9	25	0 00	30

TABLE 8

Replacement of calcium in bone by lead

NUMBER	INITIAL MOLAR CONCENTRATION 1 b × 1000	MOLS OF Pb ABSORBED × 1000	MOLS OF CALCIUM LIBERATED × 1000	MOLS OF Pb ABSORBED / MOLS OF Ca LIBERATED
1	1 38	1 36	1 09	1 25
2	2 07	2 05	1 82	1 12
3	2 78	2 66	2 08	1 28
4	3 45	3 22	2 28	1 41
5	4 14	3 80	2 41	1 57
6	5 52	4 36	2 74	1 59

pH = 7 4 Volume = 175 cc

of lead by crushed and ignited bone on the one hand, and by decalcified bone and kelp charcoal on the other The two latter apparently *adsorb* the lead while the first two replace calcium chemically by lead The microscopic appearance of the absorbed lead is different in the two cases Sections show that lead is deposited as a dense, compact layer on the surface of ignited or normal bone, while in decalcified bone it penetrates deeply The variations in degree

of absorption of lead by these four substances are recorded in table 10 and figure 12.

The absorption of lead by cartilage As noted above, cartilage has been found to take calcium from solution, and recent experiments

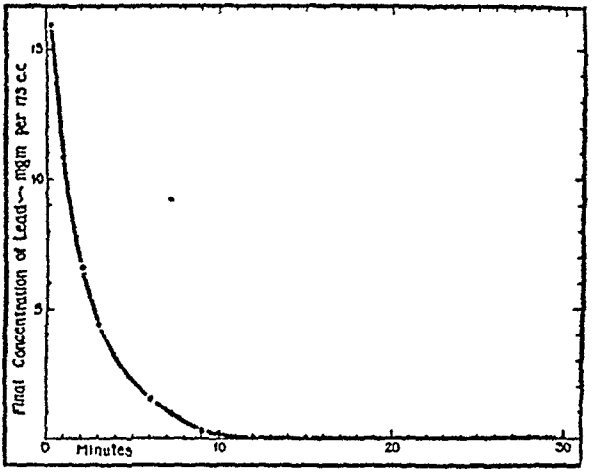


FIG 9 RATE OF ABSORPTION OF LEAD BY BONE

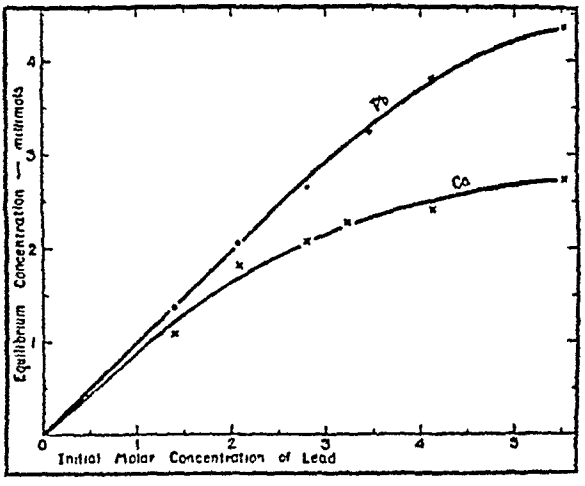


FIG 10 CHEMICAL REPLACEMENT OF CALCIUM IN BONE BY LEAD

have also demonstrated its power to remove lead from the surrounding medium The process, unlike that of the absorption of lead by bone, does not bind the lead closely and is not complete. It may possibly be adsorption rather than chemical union The quantity of lead absorbed is directly proportional to the time of contact and

varies with the hydrogen ion concentration of the solution Impregnated cartilage gives up its lead much more readily in acid solution than does crushed bone after the same treatment If the lead combined directly with the cartilaginous protein, as it evidently does, this would be expected, because lead albuminate is very soluble

TABLE 9

Effect of hydrogen ion concentration upon absorption of lead

NUMBER	INITIAL AMOUNT OF LEAD IN SOLUTION	FINAL AMOUNT OF LEAD IN SOLUTION	pH
	mgm	*mgm*	
1	125	72 3	4 0
2	125	45 4	5 0
3	125	34 8	6 0
4	125	16 8	6 8
5	125	12 3	7 2
6	125	10 8	7 4
7	125	7 8	7 6
8	125	6 5	7 8
9	125	15 1	8 0

TABLE 10

Absorption of lead by crushed, decalcified and ignited bone, and kelp charcoal

NUMBER	Pb IN SOLUTION	LEAD IN SOLUTION			
		Crushed bone	Decalcified bone	Ignited bone	Kelp charcoal
	mgm	*mgm*	*mgm*	*mgm*	*mgm*
1	25	0 00	3 72	0 00	4 77
2	50	0 40	10 09	0 00	20 45
3	75	0 89	27 64	0 00	40 12
4	100	7 23	45 48	0 77	56 06
5	125	8 05		2 96	
6	150	17 50	89 99	11 03	90 71
7	200	42 00		19 42	

in an acid medium—it dissolves at hydrogen ion concentrations slightly above pH 5 8

These experiments with crushed bone do not demonstrate that lead is taken from the circulating blood stream *in vivo* by direct chemical replacement, but they do both explain the removal of lead by bones, either dried or freshly dissected from the animal, and emphasize the sensitiveness of absorbed lead to changes in hydrogen

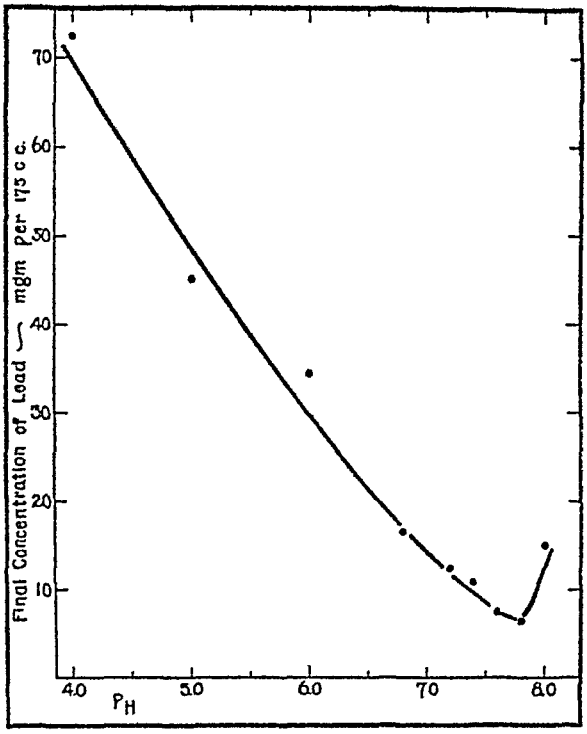


FIG 11 EFFECT OF HYDROGEN ION CONCENTRATION UPON ABSORPTION OF LEAD BY BONE

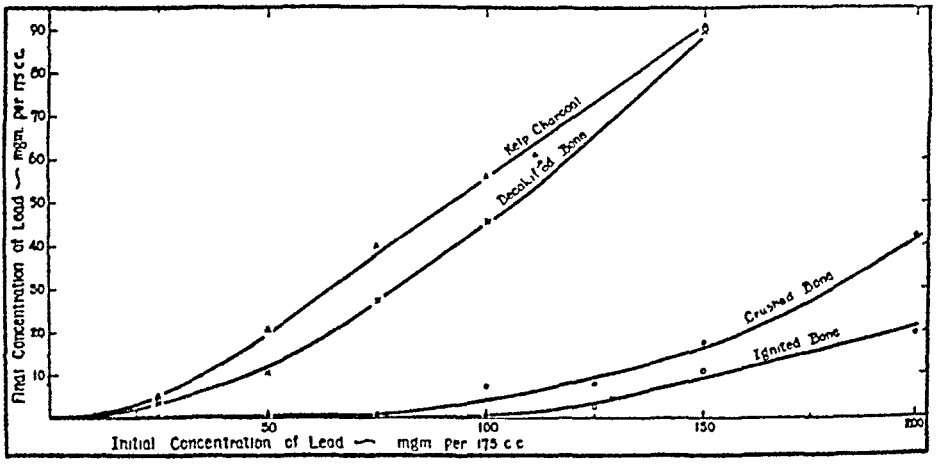


FIG 12 ABSORPTION OF LEAD BY CRUSHED, DECALCIFIED, AND IGNITED BONE, AND BY KELP CHARCOAL

ion concentration *In vitro* the deposition of lead is comparatively gross as compared with that in the bones of an animal slowly being poisoned with lead.

Although the ratio of calcium to lead in bone during life must be very much higher than during experiments *in vitro* our results probably indicate the general direction of the reactions

The data indicate that lead is slowly deposited in bone—possibly by adsorption of colloidal tri-lead phosphate When deposited this is very inert and insoluble under normal conditions, but any changes in the reaction of the tissues, either toward the acid or alkaline side, allow it to be gradually transformed into the di-lead salt which is one hundred times more soluble

Local changes of the reaction of tissues may therefore mobilize the lead within the organism—particularly if lead is deposited near the blood vessels of the bones where it can be affected by any variations in the reaction of the blood

Summary Lead is probably transported in the blood stream in the form of colloidal lead phosphate and deposited in the bone tissue as tertiary lead phosphate This is sensitive to changes in acidity, particularly to variations caused by such acids as lactic This may in part account for the fact that in the body the concentration of lead in muscle is very low and that a majority of the deposited lead is in bone tissue The sensitiveness of tertiary lead phosphate to changes in hydrogen ion concentration may be an important factor in the frequent development of acute lead intoxication following acute infection or acidosis

V LEAD ABSORPTION

Information regarding the rates and methods of absorption of lead by various routes is of great importance both from a physiological and practical point of view Industrially the main portals by which lead enters the body are the respiratory tract, the gastro-intestinal tract, and the skin The absorption of lead introduced into the subcutaneous tissue and the intraperitoneal cavity is of experimental interest

Since investigation of the problem of lead absorption was first stimulated by industrial tragedies, it is not strange that cutaneous absorption was given an important place by many early observers Even today surely the most obvious exposure of workmen to lead is by way of the skin The characteristically besmeared hands and

faces of painters and the covering of dust on the clothes and features of men handling dry lead compounds repeatedly raises the question Can lead be absorbed by the intact skin?

Manouvrier (280) in a review of the literature up to 1873 quotes among others Canuet (66) (1825), Brambilla (53) (1837), Borghi (46) (1840), Bricheteau (54) (1848), and Fievée de Jeumont (136) (1855) as supporters of the view that lead may be absorbed through the skin To Manouvrier (280), Eichhorst (110), Malherbe (276), Rousseau (392), and others further evidence of local absorption through the skin has seemed to be furnished by the frequent appearance of paralysis or arthralgia in those parts most exposed to lead These views have been persistently preserved and handed down in the literature and we find as recent a writer as Oliver (337) suggesting the possibility of cutaneous absorption of lead from cosmetics.

When experimental studies are employed, however, to test the validity of this plausible and attractive theory the results seem on the whole to be negative.

Tanquerel des Planches (453) (1839) was unable to produce symptoms of poisoning in dogs and rabbits by daily rubbing the shaven skin with various lead preparations or by the application of diachylon plasters Similarly, Sussmann (450) (1918) could produce only very slight absorption (as evidenced by the detection of small amounts of lead in the excreta and tissues) by rubbing and poulticing the shaven skin of cats with lead oleate. Rand (375) has pointed out the possible inaccuracy involved in drawing conclusions about cutaneous absorption in man from data obtained from experiments on non-perspiring animals

Nevertheless such modern authorities as Legge and Goadby (252), Meillère (293), Oliver (337), and Hamilton (188) all agree that the intact skin is practically negligible as a portal of entry for inorganic lead compounds. There is danger, however, in making too liberal generalizations from this statement Numerous records of cases of plumbism resulting from the application of lead preparations to ulcers, burned areas, skin eruptions, as vaginal douches, or eye washes, etc (356) (24) (102) (353) (56) indicate that the broken or irritated surfaces may offer a much more ready path of entrance to lead Neither can deductions as to the rate of absorption of organic

lead compounds through the skin be drawn from experiments with aqueous solutions of lead salts Many compounds which dissolve fat, as for example methyl salicylate and nitrobenzol, are known to penetrate the skin readily The general opinion that there is danger of absorption in handling *organic* lead compounds such as lead tetraethyl is apparently well founded Furthermore, the absence of cutaneous absorption should not be interpreted as a justification for any relaxation in the regulations for personal sanitation among lead workers Although the hand itself probably does not absorb lead, it may still be the means of carrying lead compounds to the mouth or nose where ready entrance to the organism is offered

A strictly chronological discussion of the theories of lead absorption is not possible because advocates of all views may be found at any given time and because absorption rarely takes place exclusively by one route except under experimental conditions Nevertheless, the common practices of sweetening wine with lead acetate (453), of cooking in lead or pewter dishes (453), of conducting public water supplies through lead pipes, and even of using lead compounds medicinally for certain intestinal disorders, made poisoning from gastro-intestinal absorption a much more common occurrence in earlier times This gave rise to the generally accepted belief, which has persisted in popular opinion until the present, that the lead which enters the gastro-intestinal tract is chiefly responsible for the symptoms of plumbism Meillère (293) (1903) holds this view and indeed as recently as 1921 in the Agenda of the International Labor Office of the League of Nations the following statement was made "The digestive canal is undoubtedly the most important path of entry the respiratory canals have only a secondary importance in this connection and even this is still contested"

Absorption through the respiratory tract The fact that lead is absorbed by the gastro-intestinal tract is doubted by no one Both experimental and accidental cases of lead intoxication have repeatedly occurred when lead could not possibly have entered by any other route Moreover Carlson and Woelfel (68) have shown that even the most insoluble lead compounds dissolve to some extent in gastric juice On the other hand, the persistent reiteration from Stockhusen's (444) time to the present, by students of the industrial lead hazard, that the

dusty trades are the most dangerous, suggests that more attention should be given to the respiratory tract as a portal of entry.

As early as 1840 Tanquerel (293) (page 89, vol 1), performed one experiment to test this point. Minium was insufflated through a tracheal cannula in a dog, and fatal lead poisoning gradually developed, and thus proved that lead had been absorbed Little attention was given to this result, however, and not until 1912 do we find another serious attempt to demonstrate experimentally that lead is absorbed through the respiratory tract At this time Goadby (165) showed that when animals were confined in an atmosphere of lead dust, toxic symptoms developed more quickly than when ten times as much lead was administered by mouth Although the possibility of simultaneous gastro-intestinal absorption was not excluded, these experiments clearly indicate that pulmonary absorption is by no means negligible.

Extensive studies of this problem have recently been made (308) In the first series of experiments designed to prove definitely that lead compounds are absorbed by the respiratory tract, the following technique was employed·

Cats were prepared by aseptically ligating the esophagus to rend swallowing impossible Suspensions of finely divided lead carbonate, oxid chromate, or sulphide in sterile physiological salt solution were then intr duced into the trachea as near the bifurcation as possible After varyi lengths of time the animals were killed and the amount of lead present in the tissues except the respiratory tract was determined

The fact that even after only a few hours significant amount lead (20 to 50 mgm) were found distributed in the various tis leaves no doubt about absorption of even the most insoluble compounds by the lungs The objection may, however, be ra that where dust is simply inhaled very much less reaches the l than when a suspension is artificially introduced (328) (254) (This is probably true, but the work of Blumgart (40) in this labor has shown that this factor does not greatly alter the rate of absor

He introduced solid lead carbonate particles into the nasopharynx prepared by means of a tracheal cannula and ligation of the eso

so that lead could enter neither the lungs nor the gastro intestinal tract After one to three days had elapsed these animals were killed and the quantity of lead present in all the tissues exclusive of the head was determined Here again evidence of the rapid absorption showed that lead dust needs merely to reach the nasal passages in order to gain ready entrance into the organism

These somewhat drastic experiments prove conclusively that lead is absorbed by the entire respiratory tract, but the experimental subjects were in too abnormal a condition and the duration of the experiments was too limited to allow a comparison of the course of events in the intoxications following gastro-intestinal and respiratory absorption Consequently another series of experiments was carried out in which various lead compounds were introduced into the respiratory tract of normal animals This was accomplished with very little respiratory disturbance by inserting a needle through the unbroken skin into the lumen of the trachea and injecting approximately 2 cc of a suspension of lead carbonate or other salt in physiological saline Undoubtedly some lead was swallowed in these cases so that there was some gastro-intestinal absorption, but the development of symptoms was so much more rapid in these animals than in the cats which had received far greater quantities of soluble lead by mouth that the relative danger involved in the two forms of exposure was clearly demonstrated Lead lines appeared within four or five days and such typical signs of plumbism as loss of appetite, constipation, weakness and nervous symptoms developed rapidly If the insufflation of dust was repeated, intoxication soon became fatal

A comparison of the quantities of lead absorbed into the organism by different routes is given in table 11 The fact that during long experiments the disproportion between the quantities of lead retained after absorption from the respiratory and gastro-intestinal tracts decreases, it makes evident that, if time allows, excretion becomes very important When lead enters the body by way of the respiratory tract, both absorption and excretion involve transportation in the systemic blood, while lead entering through the gastro-intestinal tract may be excreted without being absorbed, or after absorption may be caught by the liver and excreted in the bile without ever reaching

the systemic circulation Thus a large proportion of the lead absorbed from the gastro-intestinal tract is confined to a region where it causes relatively little damage

The course which lead follows from the time of entrance into the organism until it is excreted or stored explains in part the great danger involved in exposure to lead dust, and makes it quite evident that the dusty trades are the most hazardous because they allow absorption through the respiratory tract Figures 13 and 14 illustrate diagrammatically the course of lead within the body. The black circles

TABLE 11

Comparative rates of lead absorption by different routes

NASO-PHARYNX (FROM BLUMGART)			LUNGS			GASTRO-INTESTINAL TRACT		
Pb insufflated as $PbCO_3$	Duration of experiment	Pb in body exclusive of head	Pb insufflated as $PbCO_3$	Duration of experiment	Pb in body exclusive of lungs	Pb fed as lead acetate solution	Duration of experiment	Pb in body exclusive of gastro-intestinal contents
mgm	*hours*	*mgm*	*mgm*	*hours*	*mgm*	*mgm*	*days*	*mgm*
480	18	8 0	250	19	26 6	2,000	9	5 8
410	24	24 6	250	24	16 7	2,580	18	24 8
{180 {300	30–36	30 6	250	42	72 4	2,800	30	37 1
{300 {470	30–36	24.3	250	72	71 9	7,110	67	23 9
			250	90	35 1	11,400	99	86 3
			250	94	42 6	6,280	124	88 9

represent areas of injury, the hollow circles comparatively resistant areas

Gastro-intestinal absorption. Though the stomach is chiefly responsible for the solution of swallowed lead particles, there is no evidence that the absorption of lead is confined to any special part of the gastro-intestinal tract Some difference of opinion does, however, exist as to the region where absorption is most rapid.

Meillère (293) and Brouardel (56) believe that any mucous surface—hence the entire alimentary canal—can absorb lead. Legge and Goadby (252) state that absorption is very slight in the stomach and takes place most

rapidly in the upper part of the small intestine Somewhat opposed to this view is the opinion of Harnack (200) that bile so reduces the rate of absorption that it becomes very slight in the part of the intestine where the bile enters

The presence of food, and more especially of milk, in the gastro-intestinal tract has been generally supposed to decrease greatly the rate of lead absorption This theory is based upon the arguments

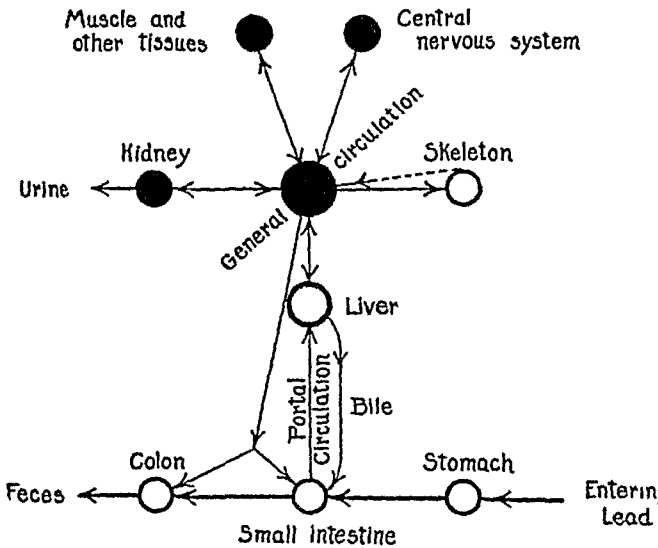


FIG 13 DIAGRAM ILLUSTRATING THE COURSE OF LEAD WITHIN THE BODY AFTER ENTRANCE FROM THE GASTRO-INTESTINAL TRACT

The solid black circles represent areas susceptible to injury, the hollow circles comparatively resistant areas

(*a*) that solution of solid lead compounds in the stomach is retarded by the reduction of acidity, and (*b*) that the food itself unites with lead to form comparatively insoluble compounds

In the course of experimental work in this laboratory, lead acetate solution mixed with 75 cc of milk was administered to one group of cats while

to others the same doses (50 mgm of lead per kilo of body weight) of lead were given in water twelve to twenty hours after the last food had been taken. In both cases lead was given on alternate days until death As the figures in table 12 indicate, there was no consistent nor significant difference between the quantities of lead retained in the body by the two groups of animals

Thus, although the general belief in the value of a milk diet as a preventive of lead poisoning is undoubtedly well founded as will be

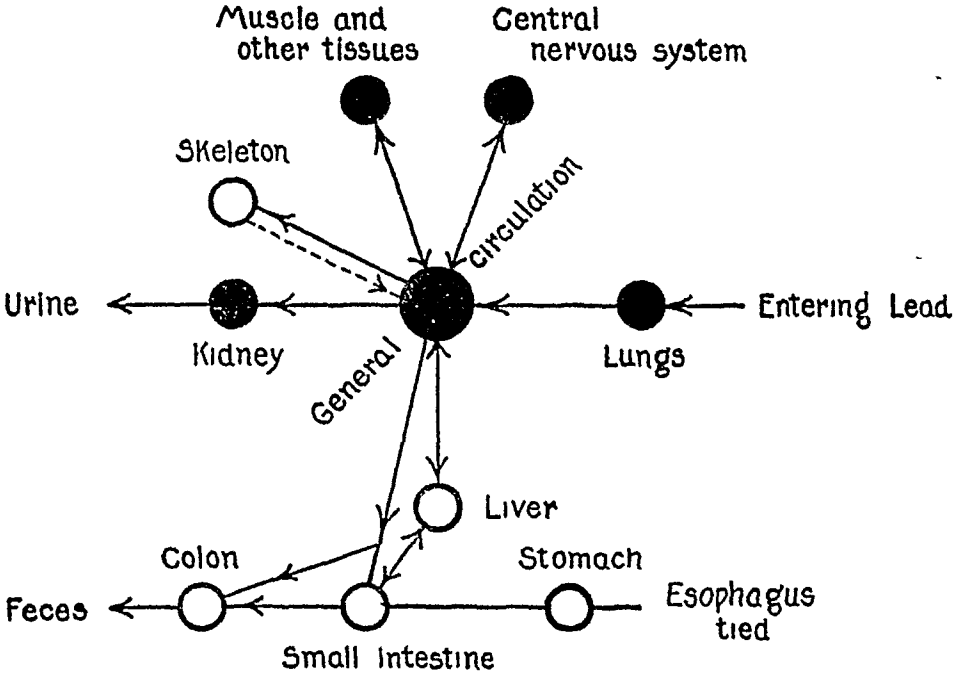


FIG 14 DIAGRAM ILLUSTRATING THE COURSE OF LEAD WITHIN THE BODY AFTER ENTRANCE FROM THE LUNGS

The solid black circles represent areas susceptible to injury, the hollow circles comparatively resistant areas

shown later (section IX, page 101), the beneficial effect of milk is due to its dietary properties rather than to any effect on the absorption of lead

The exact processes involved in absorption of lead from either the respiratory or gastro-intestinal tract are not clearly understood, but certain observations have been made which point to the type of physiological reactions which occur In the lungs the presence of poly-

morphonuclear leucocytes and mononuclear phagocytic cells containing lead indicates that phagocytosis probably aids absorption The invariable storage of lead in the skeleton as the phosphate, no matter in what form it was administered, furnishes ample proof of true solution during absorption or transportation Even with the extremely sensitive test which Cazeneuve (73) devised for chromic acid, no chromate could be found in the bones after insufflation of lead chromate (308) To permit such solution a relatively high degree of acidity is necessary Obviously, ingested lead can be dissolved in the acid of the gastro-intestinal tract (68), but in the lungs some complicated mechanism must be involved in producing sufficient acidity Perhaps the ready penetration of CO_2 through cell membranes (226) (227) permits the hydrogen ion concentration of the cells to increase to the point where solution can take place

TABLE 12

Effect of milk on the rate of gastro intestinal absorption of lead

WITH MILK				WITHOUT MILK			
Number of cat	Duration of experiment	Total lead administered	Total lead found in tissues	Number of cat	Duration of experiment	Total lead administered	Total lead found in tissues
	days	*grams*	*mgm*		*days*	*grams*	*mgm*
10	43	3 78	16 00	148	18	2 58	24 85
381	55	3 74	46 48	12	30	2 70	38 07
179	95	4 72	23 00	7	66	7 14	28 89
144	99	11 40	86 33	149	124	6 28	88 99

Subcutaneous absorption Subcutaneous injections have frequently been used as an experimental means of inducing very gradual absorption of lead Straub (448) and Erlenmeyer (122) seem to have been most successful with this method and have frequently produced in cats a fatal and very chronic type of plumbism In this laboratory the subcutaneous injection of 220 mgm of lead as carbonate failed to produce any toxic effects except the appearance of the lead line in a few cases Several times, however, local necrosis resulted On the whole, the method was quite unsatisfactory in our hands Some absorption of lead from the subcutaneous deposit did, however, occur for several weeks after the injection, for the body tissues, exclusive of the immediate region of the deposit, were found to contain

lead (see table 13) The lead absorbed is of course represented not only by the amount found in the tissues but also by the quantity excreted. This is indicated by the discrepancy between the amount injected (220 mgm) and the amount recovered Lead absorbed from such a deposit, like that from the lungs, enters directly into the general circulation, but in these cases so gradually that the animals remain in good condition and toxic symptoms develop very slowly if at all, despite a definite absorption of lead

A point of practical interest in this connection is the possibility of lead absorption from bullets embedded in the tissues The literature on this subject is rather contradictory

TABLE 13

Analysis of tissues of cats after lead absorption from subcutaneous tissue

NUMBER	DURATION OF EXPERIMENT	AMOUNT OF LEAD INJECTED	UNABSORBED LEAD IN DEPOSIT	LEAD IN TISSUES
	days	*mgm*	*mgm*	*mgm*
1	132	220	110 45	25 72
2	149	200	157 99	29 20

Lewin (262), Leoper and Verpy (269), and Disselhorst and Schneider (99), and Oliver (339) may be mentioned among those who have reported toxic symptoms produced by the lead from bullets Bonhoff (43), however, who has done the most recent and extensive work on the subject, concludes that absorption of lead from embedded bullets is very rare.

Summary. The route by which lead enters the organism most rapidly is the respiratory tract Hence, the exposure to be most guarded against is the inhalation of finely divided particles Next in danger comes the continued ingestion of lead compounds, and last of all the possible slow cutaneous absorption in persons with tender, irritated, or broken skin

VI. DISTRIBUTION AND STORAGE OF LEAD

Storage of lead in the organism is suggested by the characteristic latency and frequent recurrence of chronic plumbism Ever since analytical methods have been available, accurate chemical information about the mechanism of these processes has been sought. As

a result, the literature since 1840 contains a tremendous amount of data on the lead content of the tissues and excreta of men and animals during plumbism But because most of the investigations include examinations of only one or two tissues most directly involved in the clinical symptoms, a review of these data adds nothing to a real understanding of the fate of lead in the organism The few more extensive studies deserve recapitulation because they provide an outline of what was known about the distribution and storage of lead before this investigation

In 1861, Gusserow (182) published a most suggestive paper which should have influenced later work more than it apparently has By means of a somewhat crude electrolytic method he analyzed the various tissues of poisoned rabbits, and found by far the highest concentration of lead in the bones Muscles, kidney, liver, and central nervous system came next in order In discussing his observations Gusserow suggested an analogy between the storage of lead and calcium in the skeleton and hinted that changes in the organism as a whole might give rise to migrations of lead as well as of calcium to and from the bony deposit This suggestive idea was ignored by Gusserow's contemporaries, but much attention was focused upon his statement that the muscles contain a relatively higher concentration of lead than do the other soft tissues On the basis of this work and without further experimentation, Rosenstein (390), Hitzig (218), and others soon proceeded to explain the entire syndrome of chronic plumbism in terms of lesions produced by lead on muscles Heubel (213), who made an extensive chemical study of the distribution of lead among the tissues by means of the gravimetric sulphate method, was opposed to this view Like Gusserow, he found the greatest concentration of lead in the bones, but observed that the central nervous system contained much more than did muscle He argued that because of its chemical affinity for lead and its susceptibility to toxic effects, the nervous system must suffer lesions which are at least partly responsible for the symptoms of lead poisoning Similar conclusions were reached by Ellenberger and Hofmeister (113) in their study of sheep poisoned by lead

A very thorough and critical study of lead poisoning was carried out by Prevost and Binet (368) in 1889 Their quantitative data were limited by the fact that in many cases rats and guinea pigs were used as experimental subjects The individual tissues of these small animals contained too minute amounts of lead to be measured accurately by the gravimetric

chromate method of analysis which these authors employed They did, however, point out the differences in distribution of lead during acute and chronic plumbism and showed that in acute cases of poisoning following gastro-intestinal absorption the liver contains the largest quantity of lead, with kidney and bone next in order of concentration In more chronic cases they observed that the concentration of lead in the skeleton is much higher than that in any other tissue They believed that the lead exists in the bones as the phosphate and that it can be retained in this form indefinitely.

In 1903 Meillère (293) published a large mass of data on the amount of lead in human tissues during lead poisoning Unfortunately he merely reports the limits of concentrations found in the various tissues in all his collected cases, and thus renders a comparison of individual pictures impossible Nevertheless, in his discussion, Meillère emphasizes the differences in distribution at various stages of the disease and reports that in old chronic cases there is a progressive selective localization of lead in the bones and keratinous tissue. This he considers is brought about by a kind of internal excretion of the lead to a harmless deposit The possibility of its mobilization is not discussed in his work

The innocuousness of the comparatively large amounts of stored lead as contrasted with the marked toxicity of smaller quantities of lead in the process of absorption has been somewhat explained by the work of Straub (447) (448) and Erlenmeyer (121) (122) They poisoned animals fatally by subcutaneous administration of lead carbonate and by means of the gravimetric sulphate method determined the lead present in the animals and excreta They found that comparatively little lead was retained in the tissues. Much of the injected lead was, however, absorbed and recovered from the excreta During transportation, toxic symptoms developed which proved fatal in many cases From these data Straub and Erlenmeyer concluded that the effects of lead are produced not by lead deposited at the site of injury, but rather by the "lead stream"—that is, by the small amount of soluble lead actually carried in the blood They argued that while the amount of lead comprising this "stream" is too small to be effective in a single dose, the influence of innumerable subminimal doses could eventually cause pathological changes in certain sensitive tissues

An attractive theory to explain the latency and recurrence of chronic plumbism could be constructed by assembling the suggestion of Gusserow, the chemical data furnished by Heubel, Prévost and Binet, Meillère and others, and these suggestions of Straub and

Erlenmeyer It is possible that mobilization of the lead accumulated and stored in the bones might produce a "lead stream" of sufficient concentration to evoke toxic symptoms But since these various investigations were carried out by such different and often inaccurate methods and included such varied types of lead poisoning, the proper place of each work in completing the picture of the fate of lead in the organism can be ascertained only after the problem has been studied by an accurate and uniform method This has recently been done in this laboratory (307) (308) (309)

"Normal lead" Before an evaluation can be made of the significance of the quantities of lead found in various tissues, it is necessary to determine whether lead is commonly present in the body As Chevreul (80) and Devergie (96) long ago pointed out, the term "normal lead" is poorly chosen Lead is not an essential constituent of any human tissue but it may be present accidentally if the customary activities and diet of an individual allow it to enter the organism There is only a quantitative difference between so-called "normal lead" and that found in cases of lead poisoning It is, therefore, important to determine how much lead is likely to be absorbed by individuals living a usual normal life at a given time and place The following figures for natural lead, published in 1840 by Devergie, although probably inaccurate because of the methods of analysis, have historical interest

SOURCE OF TISSUE	LEAD FOUND AS $PbSO_4$ IN TOTAL ORGAN
	gram
A new born infant, intestinal tube	0 001
Child eight years old, stomach	0 004
Child four years old, intestinal tube	0 025
Adult healthy woman, stomach	0 020
Adult healthy woman, intestines	0 040
Adult man, intestines	0 031
Adult man, gall bladder and bile	0 003
Sick woman, intestines	0 010
Sick woman, brain (1 pound)	0 006

At about the same time Legrip (253) and Millon (305a) found lead generally present in the blood and tissues of normal individuals

The practice of sweetening wine with lead acetate, the common employment of pewter dishes and the indiscriminate use of lead pipes, and the medicinal use of lead compounds for certain intestinal disorders can readily explain the figures obtained at this early date. As more care was gradually exerted to obtain pure food and drinking water, the amount of "normal lead" would be expected to decrease Meillère's (294) figures indicate that this occurs, for in 1903 he found small traces of lead occasionally, but by no means universally, in the tissues of individuals who were not industrially exposed to lead and showed no symptoms of plumbism This was most frequently found in the hair, nails and bones where it appeared to be stored as a harmless deposit

Since previous investigations as well as recent work in this laboratory have demonstrated that the lead retained by an apparently normal individual is held almost exclusively by the skeleton, it has seemed reasonable to test specimens of bone for "normal" lead Table 14 shows the concentrations found in twenty-six specimens of human bone obtained from patients with no symptoms of plumbism and no recent industrial exposure to lead In seven cases lead was detected. Of this number four patients were found to have had a definite history of exposure at some previous time; in one case this lead had been retained at least nine years without producing any toxic symptoms The other three gave no history of known exposure to lead but possible exposure might readily have been overlooked Nineteen of the bones analyzed contained no lead Most of these were taken from old persons who had had more opportunities for exposure than would younger individuals. Our figures indicate that in this vicinity foods, public water supplies which largely run through lead pipe, and the usual activities of daily life cause no retention of lead in the body. (See Addendum)

Distribution of lead in experimental lead poisoning. Any study of the distribution of lead in the body must include only cases with accurately known histories of exposure to lead and must be based upon the analyses of all the body tissues from different types and at different stages of the disease if it is to do more than contribute to the existing confusion Data thus obtained would serve as a basis for the interpretation of some of the figures already reported

in the literature, as well as for the explanation of cases in which less complete chemical studies are possible To obtain information of this kind, the use of experimental animals is, of course, necessary

After some preliminary trials with other species in this laboratory, cats were found to be the most satisfactory subjects for such an investigation Although readily poisoned by lead introduced through any path of entry, they are sufficiently resistant to the intoxication to allow the development of typical chronic plumbism They are, also of such a size that the entire body may be completely analyzed,

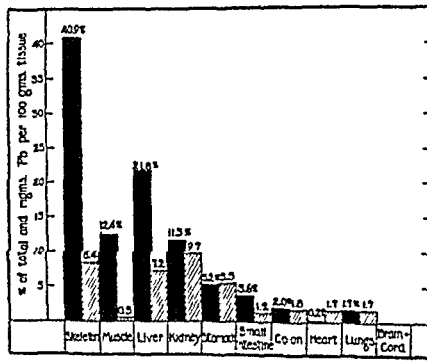


FIG 15 DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN THE TISSUES OF CAT D

See table 15 Solid black portions represent percentages of total lead in body Cross-hatched portions represent milligrams of lead per 100 grams of fresh tissue

and consequently both the percentage of total lead retained and the concentration per unit weight may be determined for each tissue A few experiments, however, carried out with dogs, rabbits, and hens show the same distribution of lead as reported here for cats

Effect of the portal of entry on distribution of lead The contradictory figures for the distribution of lead which are reported constantly by different investigators, suggest the possibility of some relationship between the portal of entry and the localization of lead in the body Harnack (200) emphasized this point some time ago and offered as an explanation for the different findings reported by

TABLE 14

Analysis of normal human bones

NUMBER	SOURCE OF SPECIMEN	WEIGHT OF SPECIMEN	LEAD FOUND	LEAD PER 100 GRAMS	SEX	AGE	BRIEF HISTORY
		grams	*mgm*	*mgm*			
1	Femur	152	0 00	0 00	Male	60	Tinsmith for twenty-five years Leg amputated for diabetic gangrene
2	Tibia	210	0 00	0 00	Female	57	Housework until three years before Amputation of lower leg for arteriosclerotic gangrene
3	Ribs	51	0 00	0 00	Female	27	Clerk Rib resected for unilateral pulmonary tuberculosis
4	Ribs	75	0 00	0 00	Male	31	Weaver in cotton mill for three years Resection of ribs for chronic empyema
5	Tibia Fibula Tarsus	332	0 87	0 26	Male	11	Traumatic amputation of leg Only known exposure to lead was careless use of paint two months before
6	Tibia Fibula Toe	225	0 00	0 00	Male	63	Weaver Amputation for arteriosclerotic gangrene
7	Lower leg	350	0 00	0 00	Male	58	Fireman Amputation for osteomyelitis
8	Vertebra	60	0 00	0 00	Male	44	Occupation unknown Lobar pneumonia
9	Tibia Fibula	225	0 00	0 00	Male	15	Stock clerk for three months Amputation for sarcoma
10	Vertebra	63	0 74	1 18	Male	60	Veterinary Until fifteen years before death had used drinking water from well contaminated with lead
11	Tibia	351	0 00	0 00	Male	69	Night watchman for six months Usual occupation unknown Amputation of left leg for arteriosclerotic gangrene
12	Tibia	208	0 00	0 00	Male	50	Tailor Amputation of a painful stump for osteitis

13	Tibia	162	0 00	0 00	Female	66	Housewife Amputation of unhealed stump for diabetic gangrene
14	Foot	245	0 22	0 09	Male	38	Brakeman on railroad for twelve years, except for occasional painting Painter seven years previous to present occupation Traumatic amputation
15	Tibia	362	0 00	0 00	Male	75	Occupation unknown
16	Vertebra	61	0 72	1 18	Female	58	Demonstrator and saleswoman Frequently painted pictures, furniture, and interior of her house Syphilis of stomach and cirrhosis of liver
17	Tibia Fibula Foot	810	0 00	0 00	Male	62	Mill worker Amputation for arteriosclerotic gangrene
18	Tibia	205	0 00	0 00	Male	52	Occupation unknown Traumatic amputation
19	Femur	420	0 00	0 00	Male	58	Coal heaver for twenty years Amputation
20	Radius and ulna	245	0 00	0 00	Male	45	Laborer Traumatic amputation of forearm
21	Femur	235	0 00	0 00	Male	15	School boy Amputation for septicemia
22	Tibia	340	7 08	2 08	Male	60	Freer in shoe factory for thirty years No known exposure to lead Amputation for diabetic gangrene
23	Femur	300	0 00	0 00	Male	64	Mechanic for fifteen years Amputation for arteriosclerotic gangrene
24	Femur	217	0 00	0 00	Male	33	Twelve years in rubber plant, spreader and curer of rubbers Amputation for progressive thromboangiitis
25	Tibia	132	1 21	0 90	Female	67	Housewife No known exposure to lead Diabetic gangrene
26	Tibia	335	5 62	1 68	Female	65	Cook Amputation for diabetic gangrene Only known exposure to lead was use of lead water pipes for many years

Annuschat (9) and Lehmann (257) the fact that Annuschat had fed lead by mouth while Lehmann had introduced it into the general circulation by means of a subcutaneous injection

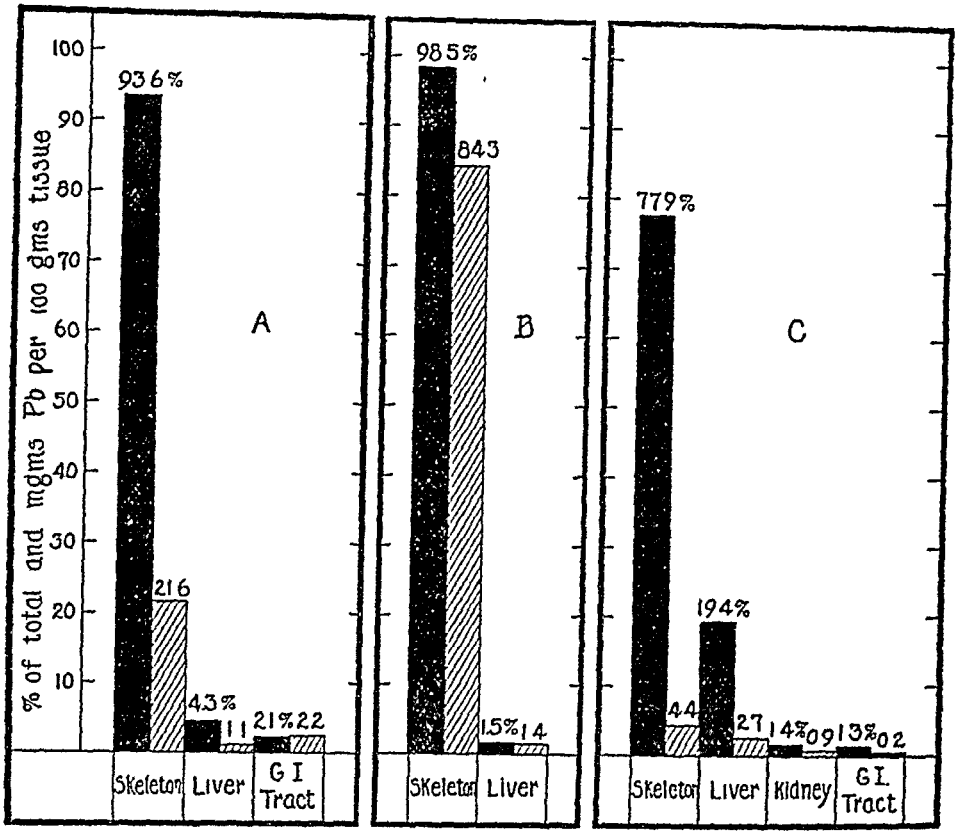


FIG 16 DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN CATS 2, 5 AND 13 (TABLE 16)

Solid black portions represent percentages of total lead in body exclusive of lungs. Cross-hatched portions represent milligrams of lead per 100 grams of fresh tissue No lead was determined in tissues not represented in the figure

A, Cat 2, nineteen hours after insufflation of 5 cc of lead carbonate suspension Total lead in body exclusive of lungs, 26 62 mgm

B, Cat 5, forty-two hours after insufflation of 4 cc of lead carbonate suspension Total lead in body exclusive of lungs, 72 40 mgm

C, Cat 13, ninety-six hours after insufflation of 2 cc of lead carbonate suspension Total lead in body exclusive of lungs, 12 71 mgm

A comparison of the various results obtained in this laboratory during a study of the distribution of lead in the tissues of animals which had received lead by different routes up to the time of death,

throws more light on this problem (307) (308) For ease of comparison the experiments may be classified as follows

Group A Absorption by gastro-intestinal tract The cats in this group were poisoned by a solution of lead acetate administered by stomach tube in doses of 50 mgm of lead per kilo of body weight every other day

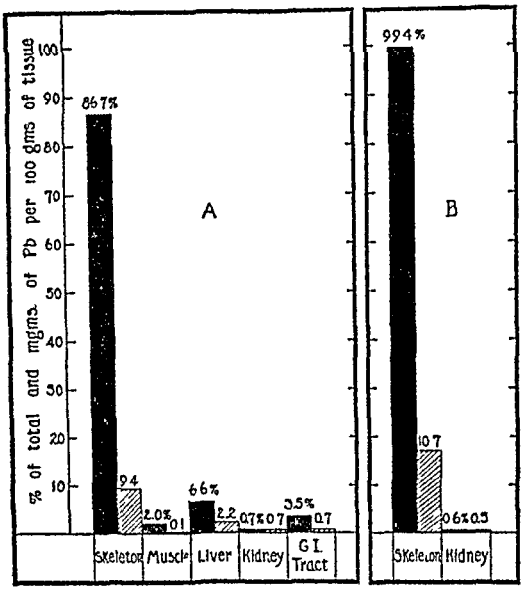


FIG 17 DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN CATS A AND B FOLLOWING THE SUBCUTANEOUS ABSORPTION OF LEAD

See table 17 Cat A killed 132 days after the injection of 220 mgm of lead Total lead in tissues 25 72 mgm

Cat B killed 149 days after the injection of 220 mgm of lead Total lead in tissues 29 20 mgm

Group B Absorption by respiratory tract For these experiments the animals were prepared by ligating the esophagus in an aseptic operation While the animal was still anesthetized approximately 200 to 250 mgm of lead were introduced into the trachea as a suspension of finely divided lead carbonate in physiological salt solution In some cases this insufflation

TABLE 15

Collected distribution figures for ten animals poisoned by gastro-intestinal route

ANIMAL	WEIGHT		LENGTH OF EXPERIMENT	TOTAL LEAD		PERCENTAGE DISTRIBUTION OF ABSORBED LEAD IN TISSUES											
	Start	End		Given	In body	Skeleton	Muscle	Liver	Kidney	Stomach	Small intestine	Colon	Heart	Lungs	Brain and cord	Spleen	Omentum
	kgm	*kgm*	*days*	*grams*	*mgm*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Rabbit A	3 6	3 4	9	1 09	5 85	47 3	0 0	29 7	6 0	5 1	4 0	5 5	1 4	1 4	0 0		
Cat C	3 0	2 2	12	1 35	12 86	81 5	5 0	6 3	2 1		3 2*			1 9	0 0		
Cat D	4 3	3 4	18	2 58	24 85	40 9	12 4	21 8	11 3	5 2	3 6	2 0	1 2	1 7	0 0	0 0	0 0
Cat E	3 4	1 8	30	2 80	37 08	84 4	10 3	0 6	0 5	1 4	1 3	1 5	0 6	Trace	0 0		
Cat F	3 3	2 4	43	3 78	11 24	69 9	6 4	6 6	1 4	3 9	2 6	2 2	3 8	0 0	3 2		
Cat G	4 6	2 8	55	3 74	46 48	65 8	1 7	7 9	5 4	7 0	1 2	0 4	7 0	1 4	0 4	0 5	0 6
Cat H	4 0	2 4	67	7 11	23 90	39 0	20 5	14 7	13 6	5 0	0 8	3 0	0 8	1 8	0 6		
Cat I	3 7	1 6	95	4 72	23 00	47 2	4 5	20 6	6 1	2 2	6 1	2 5	0 9	1 8	1 2	1 6	
Cat J .	5 5	4 5	99	11 40	86 33	77 2	12 8	7 4	0 3	0 2	0 2	0 6	0 3	0 3	0 1		0 6
Cat K .	3 4	1 8	124	6 28	88 99	85 5	4 0	3 6	2 4	1 5	0 1	1 6	0 4	0 2	0 2		

* Gastro-intestinal tract as a whole

was accomplished by means of a syringe and a small tube introduced through the mouth and nearly to the bifurcation of the trachea, and in others through a needle inserted in the wall of the exposed trachea No attempt was made to feed the animals but fluid was introduced daily by intraperitoneal injections of physiological salt solution That absorption continued until death was demonstrated by the presence of unabsorbed lead carbonate in the lungs at autopsy

Group C Subcutaneous absorption In this series of experiments approximately 220 mgm of lead as carbonate was introduced under the skin of the back The remainder of this injection which was unabsorbed after death provided evidence that absorption had been continuous until the animals were killed

Figures 15, 16 and 17 show diagrammatically the distribution of lead typical for Groups A, B, and C, respectively The analytical data for several animals of each type are presented in tables 15, 16 and 17

Superficially, the points of similarity in these pictures are (*a*) the high percentage of the total retained lead held in the skeleton, and (*b*) the relatively uniform order of concentration of lead in the soft tissues of the different groups (Traces of lead could as a rule be detected by the microchemical test in the tissues not shown in the diagram of the animals of Groups B and C) The most obvious differences, on the other hand, are (*a*) the markedly higher concentrations of lead in the liver and washed gastro-intestinal tract of the animals of Group A, and (*b*) the larger amounts of lead in the tissues of Group B after an absorption period of *hours* as contrasted with the quantities retained by Groups A and C after *days* or *weeks* of absorption

From a closer study it becomes apparent that in every case, disregarding for the moment the lead in the liver, the same disposal is made of lead which reaches the general circulation The most striking feature of this distribution appears at once to be the great affinity between lead and bone The other tissues, in contrast, retain only such small amounts of lead as might be found in any tissue receiving a supply of blood in which lead was transported In general the relative concentration of lead in the soft tissues is in direct ratio to their vascularity

TABLE 16

Distribution of lead in cats which have received solid lead carbonate by lung, the gastro-intestinal tract being excluded as a portal of entry

NUMBER	WEIGHT OF CAT	DURATION OF EXPERIMENT	AMOUNT OF SUSPENSION INSUFFLATED	TOTAL LEAD IN BODY*	PERCENTAGE DISTRIBUTION OF TOTAL LEAD				REMARKS
					Skeleton	Liver	Gastro intestinal tract	Kidney	
	kgm	*hours*	*cc*	*mgm*	*per cent*	*per cent*	*per cent*	*per cent*	
1	1 5	12	5 0	2 62	86 6	13 4	Trace	0 0	Poor condition Bronchopneumonia
2	3 5	19	5 0	26 62	93 6	4 3	2 1	0 0	Fair condition Some congestion of lungs 150 cc 0 9 per cent NaCl solution given intraperitoneally
3	3 2	24	5 0	16 72	90 5	6 0	3 5	0 0	Very good condition 150 cc Ringer solution intraperitoneally
4	1 6	24	5 0	18 43	100 0	0 0	0 0	0 0	Poor condition. Much respiratory disturbance No fluid given
5	3 6	42	4 0	72 40	98 5	1 5	0 0	0 0	Developed bronchopneumonia No fluid given
6	3 5	48	5 0	16 22	96 1	3 9	0 0	0 0	Many hemorrhagic areas in lungs 100 cc Ringer solution intraperitoneally
7	2 0	56	5 0	14 75	92 0	8 0	0.0	0 0	Lungs in fair condition 75 cc Ringer solution and 100 cc 5 per cent glucose solution intraperitoneally
8	3 0	72	5 0	71 87	98 3	1 7	0 0	0 0	Lungs in good condition 640 cc Ringer solution intraperitoneally
9	2.1	80	5 0	14 97	69 2	24 7	6 0	0 0	Developed distemper Right lung pneumonic Left lung contained lead deposit Otherwise normal
10	3 0	90	5 0	28 95	80 6	19 4	0 0	0 0	Right lung showed marked congestion Left lung fairly normal 450 cc Ringer solution intraperitoneally
11	3 1	90	5 0	35 08	70 1	24 2	5 7	0 0	Lungs fairly normal 150 cc Ringer solution intraperitoneally
12	3 3	94	5 0	42 64	89 0	10 3	0 7	0 0	Pneumonia developed on last day. 550 cc Ringer solution intraperitoneally

13	2 8	96	2 0	12 71	77 9	19 4	1 4	1 3	Infection at site of wound Lungs in good condition 1055 cc Ringer solution during experiment Blood CO_2, 20 9 volume per cent at death
14	3 1	120	1 5	26 32	93 9	4 1	1 4	0 8	Lungs in very good condition 950 cc. Ringer solution during experiment. Blood CO_2, 30 0 volume per cent at death
15	2 5	162	5 0	26 25	87 2	3 8	9 0	0 0	Congestion of upper lobes of lungs 940 cc Ringer solution during experiment Blood CO_2, 19 2 volume per cent at death

* Exclusive of lead remaining in lungs

When the liver is included in the picture, however, a marked discrepancy is apparent between the percentages of lead retained by this organ in the different groups of experiments This can be readily explained by a consideration of the different points at which lead may enter the circulating blood when administered in different ways Lead absorbed in the gastro-intestinal tract is first carried in the portal blood to the liver, which, as is well known, removes foreign or toxic materials from the blood stream The high efficiency of the liver in removing lead from the portal blood explains the retention of so much lead in this organ A large percentage of this absorbed lead is gradually excreted in the bile (see work by Brady, section VII, page 78) This mechanism accounts for both the small amount of lead within the rest of the organism and the late appearance of toxic

TABLE 17

Distribution of lead in cats after lead absorption from subcutaneous tissue

Number	Duration of experiment	Weight of animal		Unabsorbed lead	Total lead in body	Percentage distribution of total lead					
		At start	At death			Skeleton	Muscle	Liver	Kidney	Gastro-intestinal tract	Brain and cord
	days	*kgm*	*kgm*	*mgm*	*mgm*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
A	132	2 7	3 3	110 45	25 72	86 7	2 0	6 6	0 7	3 5	0 0
B	149	3 3	4 0	157 99	29 20	99 4	0 0	0 0	0 6	0 0	0 0

symptoms following gastro-intestinal absorption A small quantity of lead does, however, escape retention by the liver and become distributed throughout the organism by the general circulation

Lead absorbed from the respiratory tract or from subcutaneous tissue, on the other hand, enters the *systemic* blood directly Under these conditions the liver receives only the lead carried in the hepatic artery and consequently can retain only an insignificant portion of the total quantity of lead absorbed Although all the tissues are bathed with lead while it is being transported, the only one to pick up and retain permanently any significant amount is the skeleton

Effect of time on the distribution of lead Obviously the next problem to be solved was whether in more chronic cases there is a more marked localization of lead in the bone Figure 18 which shows diagrammatically the percentage distribution in an animal which re-

ceived lead by mouth for a relatively long time, demonstrates that both the actual amount and the percentage of total lead retained in the skeleton are larger than in shorter experiments Since the actual quantities of lead in the other tissues change very little as the experiment progresses, they of course become an ever decreasing percentage of the total amount of lead within the body

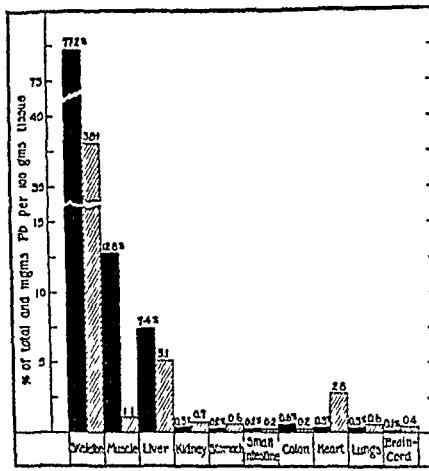


FIG 18 DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN THE TISSUES OF CAT J

See table 15 After 99 days of lead absorption by gastro intestinal tract Solid black portions represent percentages of total lead in body Cross hatched portions represent milligrams of lead per 100 grams of fresh tissue

To test whether lead may be completely localized in the bones after the cessation of absorption, cats were severely poisoned by lead administered by mouth Just before death seemed imminent the feeding was discontinued and the animals were fed on a normal diet until they gradually recovered Several weeks after the last dose of lead, when the animals were apparently in perfect health, they were killed and their tissues analyzed The localization of lead in the skeleton was found to be almost complete (fig 19)

Thus it is apparent that, from the first, the concentration of lead

in the skeleton progressively increases, while the other tissues do not retain the lead which they receive. Hence, after absorption has ceased, significant amounts of lead are to be found only in an apparently harmless deposit in the bones

Site of lead storage in the skeleton. Association of the storage of large amounts of lead in the skeleton and the frequent occurrence of marked anemia in chronic plumbism at first suggests that the lead

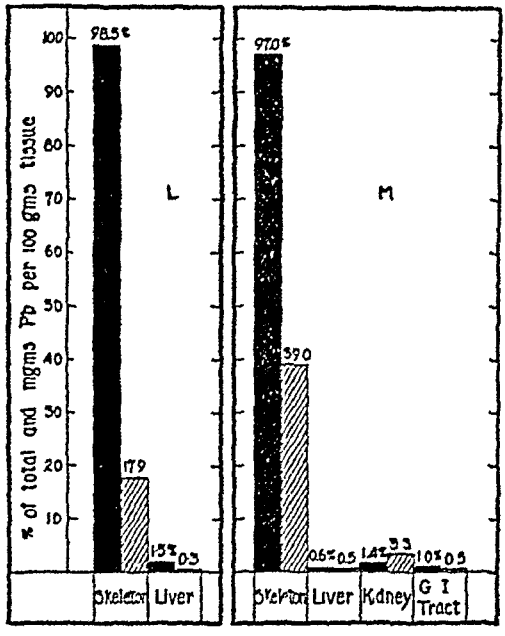


FIG 19 DIAGRAMMATIC REPRESENTATION OF THE DISTRIBUTION AND CONCENTRATIONS OF LEAD IN THE TISSUES OF CATS L AND M KILLED SEVERAL MONTHS AFTER THE LAST DOSE OF LEAD BY MOUTH

Solid black portions represent percentages of total lead in body Cross-hatched portions represent milligrams of lead per 100 grams of fresh tissue

Cat L Killed 82 days after last dose of lead Total lead in body 20 53 mgm

Cat M Killed 160 days after last dose of lead Total lead in body 97 29 mgm

by collecting in the bone marrow may interfere with the normal formation of red cells Animal studies have shown, however, that this does not occur Separate analyses of the solid shafts of bone and the marrow have shown that the marrow contains only traces of lead comparable to those in the other soft tissues, and the high concentration is to be found only in the solid portion of the bones Furthermore, the detection of high concentrations of lead in the

pneumatic marrowless bones of the wings of poisoned hens furnishes additional evidence that the marrow plays no essential rôle in the retention of lead by the bones Prévost and Binet (368) suggested without conclusive experimental proof, and Fairhall and Shaw (129) have recently demonstrated in this laboratory (section IV) that lead exists in the bones as the tertiary phosphate which is extremely insoluble and stable at the ordinary hydrogen ion concentration of the body Preliminary histological study of the bones after absorption of lead by the organism has demonstrated that lead is usually deposited in the endosteum and occasionally near the capillary walls

Discussion This experimental study of the distribution of lead has clarified several points in our general conception of the fate of lead within the organism When lead is absorbed into the general circulation it is brought into contact with every tissue in the body There is, however, no marked retention of lead except by the skeleton which paradoxically enough is the tissue apparently least harmed by this toxic agent In other words, lead is not stored at the site of injury This fact does not, however, imply that there is an absence of chemical affinity between the soft tissues and lead, but rather that lead compounds which are formed by interaction with these tissues are not retained The lead, therefore, encounters the various tissues, reacts with them if the proper physiological conditions exist, causing more or less damage, and is then not retained but eventually removed and carried on in the blood stream This course of events may conceivably be repeated until the lead reaches either some site of excretion or the skeleton Under normal conditions, a very insoluble lead compound—the tertiary phosphate—is formed in the bony tissue Since this salt is retained as a skeletal deposit, the bones gradually remove lead from the circulating blood The two processes of storage and excretion which take place during the absorption of lead continue after absorption has ceased until practically all the lead in the body is held by the skeleton While this state of affairs exists, the stored lead is apparently harmless, symptoms of plumbism disappear, and the subjects present no evidence whatever of the presence of lead within their tissues At this point, however, the old theory of Gusserow (182) comes to mind with the disquieting

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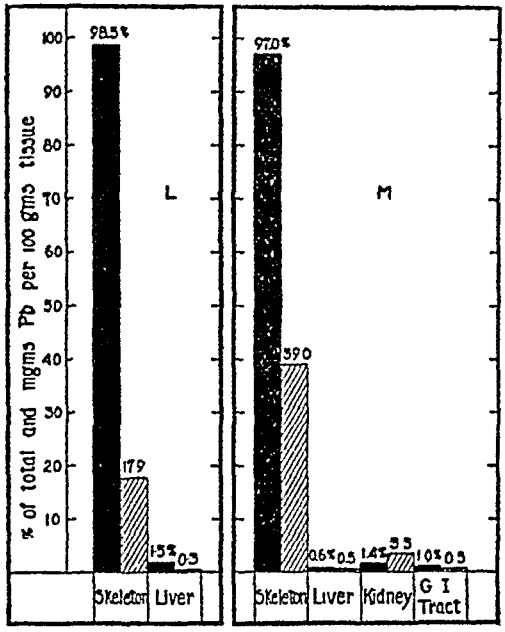

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suggestion that this desirable condition may not be permanent He advanced no proof for this but recent chemical evidence (sections IV and IX) substantiates the possibility that the skeletal deposit may be released, for a slight change toward either the acid or alkaline side of the usual hydrogen ion concentration of the organism can readily reduce the stability of the tertiary phosphate It is quite conceivable that abnormalities of diet or certain pathological conditions might bring about such changes Although this problem will be discussed in detail later (section IX) it is desirable now to mention (*a*) that the deposit of lead retained after the absorption of a lead salt is known to be rather unstable chemically, and (*b*) that this deposit is held within the body where its release would involve the danger of flooding the organism with soluble lead

The distribution of lead in the human body during lead poisoning. *Analytical studies.* These general conceptions may be applied with interest to the chemical results from human autopsies in cases representing different types and stages of plumbism Thus far it has been possible to examine in this laboratory for diagnostic purposes the tissues from seven necropsies In these few investigations we have obtained cases typical of different types of exposure to lead and representing various stages of absorption The analytical data are collected in table 18 merely to save space. The group should not be studied as a whole for each individual distribution picture and its case history should be considered separately. For complete histories reference must be made to our original report (309). Only the following brief summaries of the cases will be included here

Case I. A mentally defective child with a perverted appetite gnawed the paint from her bed for several weeks Gradually severe lead poisoning developed The symptoms were marked constipation, vomiting, loss of appetite, lead line, marked anemia and stippling of red cells, increasing weakness, tremor, drowsiness, and finally convulsions which developed and were continuous the day before death The presence of acetone and diacetic acid in the urine indicated a condition of acidosis which was further evidenced by the rather marked odor of acetone in the tissues at autopsy.

Case II. A man aged forty-two had been exposed to lead since a small child when he watched his father spray trees with arsenate of lead For

TABLE 18

The distribution of lead in human tissues

TISSUE	CASE I		CASE II		CASE III		CASE IV		CASE V		CASE VI		CASE VII	
	Conc.*	Total†	Conc.	Total	Conc.	Total	Conc.	Total	Conc	Total	Conc.	Total	Conc.	Total
	mgm.	*mgm.*	*mgm.*	*mgm*	*mgm*	*mgm*	*mgm*	*mgm.*	*mgm*	*mgm*	*mgm*	*mgm*	*mgm*	*mgm*
Liver	5 11	22 99	0 015	0 23	0 12	1 65	0 68	10 88	0 13	1 95	0	0	0	0
Kidney	1 00	0 97	0 09	0 30	0 35	0 94	2 45	7 84	0 39	0 88	0	0	0	0
Spleen	1 07	0 77	Trace	—	1 59	2 23	0	0	0	0	0	0	0	0
Pancreas	10 00	1 09	0 25	0 20	—	—	—	—	—	—	0	0	0	0
Washed gastro-intestinal tract	0 46	1 59	0 01	0 26	—	—	—	—	—	—	0	0	0	0
Heart	0 56	0 30	Trace	—	0 32	0 99	—	—	0	0	0	0	0	0
Lung	0 32	0 61	0 01	0 17	0 29	4 41	0	0	0	0	—	—	—	—
Cerebrum	0 36	2 91	0 02	0 31	0	0	0 22	3 20§	—	—	—	—	0	0
Cerebellum	0 62‡	0 65‡	0 17	0 20	0 49	0 71	0 30	—	—	—	—	—	0	0
Peripheral nerves and cord	—	—	Trace	—	—	—	—	—	—	—	—	—	—	—
Skeletal muscle	—	—	0	0	—	—	—	—	—	—	—	—	—	—
Skeleton	15 30	195 8	4 05	390 0	2 24	280 0	7 16	800 0	9 36	897 0	2 17	243 0	2 17	195 0
Blood	Trace	—	—	—	0 27	—	—	—	—	—	—	—	—	—
Bile	Trace	—	—	—	0	0	—	—	—	—	—	—	—	—
Gastro-intestinal contents	—	—	—	0 17	—	0 90	—	—	—	—	—	—	—	—
Bladder contents	Trace	—	—	—	—	—	—	—	—	—	—	—	—	—

* Conc = mgm. of lead per 100 gm of fresh tissue

† Total = mgm of lead in whole organ In many cases this figure is only approximate

‡ Medulla analyzed with cerebellum

§ Calculated for entire brain

twenty-seven years he worked in the forestry department himself, using lead arsenate as a spray, and during each spraying season had severe colic. Double wrist drop had persisted for twelve years in spite of treatment, and during the last three years there was difficulty in walking. After a month's illness death was caused by pneumonia in mid-winter several months after the last exposure to lead He had a positive Wassermann reaction

Case III. A man twenty-five years old had never been exposed to lead before working in the mixing room of a rubber company for the seven months preceding death This room was very dirty and the workmen were exposed to much dust containing lead compounds Marked constipation, lead line, abdominal pains, and occasional attacks of dizziness appeared during the last five months of life while the man was employed at this trade Nine days before admission to the hospital his left eye suddenly became blind and the left arm and leg were paralyzed. This condition was only temporary and the patient continued to work until nine days later when he fell to the floor and became very violent within a few minutes He was at once taken to a psychopathic hospital Clonic convulsions continued until he died two days later

Case IV. A man aged sixty who had been a painter for six years was killed while at work by a fall He had always been well and there had been no symptoms of plumbism.

Case V. A janitor sixty-three years old who had frequently done painting died after an illness of ten weeks, which was diagnosed as coronary thrombosis and auricular flutter There were no signs or symptoms of lead poisoning but the case was studied on account of the history of exposure to lead.

Case VI A man of fifty-one had been a stereotyper for twenty-five years. This necessitated working in a small room over vats of molten metal (lead, antimony, and tin) from which fumes rose constantly. Loss of appetite, constipation and loss of weight were noted during the last nine months of life, but these symptoms were probably not due to lead The blood showed no anemia or stippling Death was due to an eroding carcinoma of the esophagus which finally produced two sinuses, one leading into the trachea and the other into the left lung

Case VII A man aged fifty-three years had been a paint mixer for fifteen years His health had been good until about nine months before

death Such symptoms indicative of lead poisoning as abdominal cramps, pallor, lead line and a rapid loss of weight were then noted but were not severe The blood was markedly anemic, suggestive of a primary type, and no stippling of red cells could be seen Death was due probably to pernicious anemia

Discussion Correlation of these analytical data and the clinical histories demonstrates that events follow the same course in both man and animals after the absorption of lead The distribution of lead in Case I is quite similar to that in animals when lead has been administered by mouth until shortly before death, and the clinical history furnishes evidence of a similar type of exposure The high concentration of lead present in the liver is a sign that in man as in animals much of the lead carried in the portal blood is removed and retained by this organ Of the lead which escapes the liver and enters the general circulation, a high percentage is retained by the skeleton While absorption continues, however, lead is also present in the blood stream and in all the tissues of the body In Cases II and III the lead was also well distributed throughout the organism In Case II there had been no exposure to lead for about six months —a fact corroborated by the absence of any accumulation of lead in lungs or liver Probably, therefore, the acute infection—pneumonia—and the inability to take adequate food during the period of illness had mobilized from the skeletal store the small amounts of lead which were distributed throughout the organism In Case III, on the contrary, the relatively high concentration of lead in the lungs as compared with that in the liver indicates that absorption through the respiratory tract was active at the time of death Further evidence of this is offered by the general distribution of lead in the tissues and by the high concentration in the blood The truth of these deductions is borne out by the history of recent work at a very dusty trade Case IV is chiefly interesting because it probably portrays conditions typically found in lead workers without symptoms of plumbism The chief features of this case are the storage of considerable quantities of lead in the bones, the rather high concentration of lead in the liver, indicative of continued gastro-intestinal absorption, and the presence of lead in the kidney and central ner-

TABLE 19

Miscellaneous analyses of human tissues and fluids

TISSUE	WEIGHT ANALYZED	LEAD FOUND	LEAD IN 100 GRAMS	PATIENT	REMARKS
	grams	*mgm*	*mgm*		
Teeth	3 6	0 23	6 37	D M.	Under treatment for chronic plumbism Removed for several weeks from exposure to lead
	17 0	3 36	19 75	B L	Chronic lead poisoning Teeth removed after six weeks hospital treatment
	12 86	0 66	5 08	T B	During hospital treatment about one month after removal from exposure to lead
Bone graft	2 5	0	0	B L	
Bile	175 cc	0	0	J L	Duodenal lavage in case of chronic plumbism
	113 cc	0	0	S S	Duodenal lavage in case of chronic plumbism
	125 cc.	Microchemical trace		M M	Duodenal lavage in case of chronic plumbism
Spinal fluid	80 0	0 08	0 10	J T.	During an attack of acute encephalopathy
	5 0	0	0		Child with lead encephalopathy
Hair	0 50	Trace, micro +		J S.	After five months hospital treatment for chronic plumbism
	2 57	Micro +		J. T	One month in hospital after exposure to lead
	4 5	0	0	P M	After three months treatment
	5 8	Micro +		P M	Six months after admission to hospital
	1 95	Micro +		J T	Four months after admission to hospital
	5 1	0 17	3 33	D. M	Three months after admission to hospital
Saliva	50.0	0	0	M R	Case of chronic plumbism on admission to hospital

vous system—a sign that the process of transportation of lead was active despite the absence of symptoms

In Cases VI and VII, on the other hand, the lead was entirely localized in the skeleton Here absorption had not taken place for some time and apparently the stored lead had not been mobilized The distribution of lead in such cases is completely analogous to that in animals which have been killed long after exposure, significant quantities of lead are to be found only in the skeleton

A few determinations of isolated tissues and fluids from cases of plumbism have been made and seem of sufficient interest to include (see table 19) A concentration of lead similar to that found in the rest of the bony tissue is present in teeth and suggests a possible way of proving previous lead absorption Though lead was frequently detected in hair, there was no great concentration to indicate a special localization of lead in this tissue as suggested by Meillère (292)

Medico-legal significance of analytical findings Since the additional evidence furnished by the chemical analysis of tissues is frequently required to settle medico-legal questions arising in cases of plumbism, it is of interest to consider what conclusions may justifiably be drawn from the different types of distribution pictures When lead appears exclusively in the bone it must have been absorbed at some previous time, but mere storage in the skeleton is no evidence that lead was involved as a cause of death Similarly, if lead is present only in the gastro intestinal tract or its contents, it may merely have been swallowed and not absorbed On the other hand, the presence of lead in the urine or in a majority of the tissues is a sign of active transportation of lead by the blood stream This is evidence of either recent absorption or mobilization of stored lead from the bones In either case danger of the exposure of the tissues to soluble lead is obvious, and lead may then rightfully be considered at least a complicating factor in the pathological picture

Summary and conclusions Studies of the distribution of lead in the tissues of man and animals during plumbism show the same general course of events Early in lead absorption differences in distribution are found dependent upon the route by which lead has entered the organism Following absorption from the gastro-intestinal tract, the liver contains a relatively high concentration of

lead due to a retention of this toxic substance which it removes from the portal blood. From the lung or subcutaneous tissue, on the contrary, lead enters the general circulation and, hence, only a small portion of it is carried directly to the liver Once in the general circulation the lead is distributed throughout the organism Since the skeleton is the only tissue to retain permanently any significant amount of lead an ever increasing percentage of the total absorbed lead comes to be stored in the bones After absorption has ceased, this selective localization of lead becomes practically complete. While stored in such a deposit the lead is apparently harmless, since the symptoms of plumbism are noted only when lead is generally distributed throughout the organism, as a result either of recent absorption from an external source, or of mobilization of a skeletal store.

VII. EXCRETION OF LEAD

After absorption into the general circulation, lead is either stored in the skeleton or eliminated from the organism by excretion To complete our knowledge of the course which lead follows from the time it is absorbed until it finally leaves the body, it remains to consider the various excretory routes and to study the factors controlling elimination and storage Of the three paths of excretion—the alimentary canal, the kidney, and the skin—that first recognized was the gastro-intestinal tract Although the very early treatment of lead poisoning by drastic purging seems to imply a knowledge of the excretion of lead in the feces, accurate information could not be obtained until methods of chemical analysis were devised As early as 1839 Devergie (96), in collaboration with Tanquerel des Planches (453), definitely detected lead in the tissues and contents of the gastro-intestinal tract and also in the kidneys and bladder of individuals suffering from lead poisoning Since that time analysis of the excreta has frequently accompanied investigations of other phases of chronic plumbism. For the sake of clarity each of the paths of excretion will be discussed separately here

Excretion by the gastro-intestinal tract. In spite of the early recognition of the gastro-intestinal tract as an important path for the elimination of lead from the organism, there are still many features

of the mechanism which are but little understood Ever since Tanquerel's time there has been no doubt that lead is eliminated in the feces, and detailed information about the comparative activity of various parts of the gastro-intestinal tract and the exact process of excretion has gradually accumulated, until there is a general belief that the entire alimentary tract excretes as well as absorbs lead (293) (39) Blum (39) considered the lead line a sign of excretion in the saliva, Pouchet's (365) experimental observation that lead is present in the saliva of patients with chronic plumbism, even after exposure has ceased, has not been confirmed in this laboratory in a patient undergoing treatment for chronic lead poisoning Renon (382) found lead in the salivary glands of experimentally poisoned animals That lead should appear in the saliva or salivary glands while it is being transported in the blood is not surprising, although excretion by this path would be rendered quite ineffective by re-absorption of the lead in other parts of the tract

No conclusive data regarding the rate of the excretion of lead in different portions of the gastro intestinal tract are available because of the difficulty of obtaining such information in an intoxication as slow as chronic plumbism As a natural result of the early observation that following lead ingestion relatively large quantities of lead are picked up and held by the liver, excretion in the bile has received much more attention than elimination by any other route

Tanquerel's (453) attempts to demonstrate the presence of lead in bile were inconclusive Although Hermann (209) (1874) really only assumed that lead is excreted in the bile after detecting it in both the liver and the small intestine, he is generally credited with having furnished final proof The first real experimental work on the subject was contributed by Annuschat (9) (1877) He fed lead to rabbits with biliary fistulae, and found considerable quantities of lead in the bile in acute stages of poisoning, in the more chronic stages little or none, although the liver contained comparatively large amounts Lehmann (257), on the other hand, in the controversy with Annuschat found that during the chronic poisoning following subcutaneous injection, the concentration of lead in the bile was higher than that in the liver, and he believed that the liver collects the lead to be poured out in the bile Lavrand (248) (1886) likewise believed that bile is the main path of excretion for lead Harnack (200) in 1897 contributed

the information that the proportion of lead excreted in the bile depends upon the portal by which it enters the body—that it is greatest following gastro-intestinal absorption Several more recent investigators have repeated these observations without adding any really new information (293) (252) Siccardi and Roncato (428) in 1912 attempted to explain the mechanism involved in excretion of lead by the liver by a suggestion which

TABLE 20

Excretion of lead in bile and gastro-intestinal tract

NUMBER OF RABBIT	AMOUNT OF LEAD GIVEN	METHOD OF ADMINISTRATION	INTERVAL BEFORE COLLECTION	LENGTH OF COLLECTION PERIOD	INTERVAL BETWEEN INJECTION AND DEATH	TOTAL PB IN BILE	AVERAGE HOURLY EXCRETION OF LEAD IN BILE	LEAD FOUND IN GASTRO-INTESTINAL TRACT			LEAD IN THE LIVER
								Stomach	Small intestine	Colon	
	mgm		*hours*	*hours*	*hours*	*mgm*	*mgm*	*mgm*	*mgm.*	*mgm*	*mgm*
701	125 0	Jugular vein	0	9	9	1 87	0 21	—	—	—	—
702	110 5	Jugular vein	0	14 5	14 5	1 16	0 08	1 01	1 81	0	56 45
703	130 0	Jugular vein	0	4	11	0 57	0 14	0 58	0 0	1 21	39 31
704	111 6	Jugular vein	0	3	4 75	0 55	0 18	0 62	0 59	—	—
705	116 5	Jugular vein	0	8 5	8 5	1 78	0 21	0 38	1 22	0 61	35 20
706	93 2	Jugular vein	0	2 5	7	2 52	1 00	2 30	0 92	1 08	36 20
708	69 0	Jugular vein	0	4	4	1 28	0 32	0 58	1 72	0 45	37 71
722	546 0	By mouth	3	3		0 43	0 14				11 19
721	546 0	By mouth	3 5	3		0 72	0 24				
723	977 0	By mouth	3	3		0 82	0 27				
725	977 0	By mouth	2	3 5		0 09	0 03				
726	546 0	By mouth	3	3		0 11	0 04				
720	1,000 0	By mouth	18	2		0 40	0 20				

Average hourly excretion in bile for all animals, 0 23 mgm

involved impossible chemical reactions According to them, lead is brought to the liver by leucocytes, detoxicated by the Kupfer cells which reduce it to the metallic state, and in this form is excreted in the bile

In a series of unpublished experiments, Brady and others in this laboratory have studied the biliary excretion in rabbits following administration of a comparatively large single dose of lead either intravenously or by mouth The bile duct was cannulated, the bile collected for several hours, and its lead content determined From these data the average hourly excretion of lead could be calculated (see table 20) Since the bile duct was cannulated before injection, detection of lead in the gastro-intestinal tract indicated

that there was simultaneous independent excretion in the bile and by the gastro-intestinal tract In comparing the amounts of lead excreted by these two paths (see table 20) it must be borne in mind that the lead in the stomach and in the intestines was excreted during the entire interval between the injection of lead and death, while the specimen of bile examined was generally collected during a much shorter period The average rate of excretion of lead in the bile of these animals, 0 23 mgm per hour, is undoubtedly higher than in more chronic cases since the larger single dose acutely flooded the organism just before the specimens were collected But the data serve (*a*) to confirm the earlier reports of an excretion of lead in the bile, and (*b*) to demonstrate an independent excretion of lead in the stomach and intestines

Our information regarding the excretion of lead by the gastro-intestinal tract as a whole may be summarized as follows (*a*) The entire alimentary canal probably excretes lead, (*b*) the bile is an important factor in this excretion, especially following gastro-intestinal absorption, (*c*) the gastro intestinal tract is generally considered the chief route by which lead is excreted

Excretion of lead by the kidney The unreliability of the methods of chemical analysis employed, and the fact that different types and stages of plumbism were studied, undoubtedly account for the variety of opinion among earlier investigators regarding the excretion of lead by the kidney

For instance, Tanquerel (453) failed to find lead in the urine, while Melsens (297) somewhat later demonstrated its presence Lavrand (248) in 1886 reported that lead was to be found in the urine of patients with plumbism only after medication Murgia (323), on the other hand, in 1912 states that the appearance of lead in the urine is the earliest and most constant sign of lead poisoning Among the more modern authorities, Legge and Goadby (252), Oliver (337), and Meillere (293) agree that the urine in cases of chronic plumbism frequently contains lead, but that the kidney excretes far less than does the gastro-intestinal tract

Two groups of experiments have been carried out in this laboratory to furnish further correlation between the urinary and fecal excretion of lead First, a few single determinations of the daily excretion in cats by these two routes were made in order to determine their relative importance following the entrance of lead into the organism by various portals The figures,

collected in table 21, agree with the reports of earlier investigators As would be expected, large quantities of lead were excreted in the feces after administration by mouth.

As Oliver (337) (page 161), Legge and Goadby (252) (page 19), and others have pointed out, however, probably only a small percentage of this apparently excreted lead had been absorbed, and the

TABLE 21

Excretion of lead in the urine and feces of cats

NUMBER OF CAT	SPECIMEN NUMBER	DAILY EXCRETION IN URINE	AVERAGE DAILY FECAL LEAD EXCRETION FOR PERIOD INCLUDING DAY OF URINE ANALYSIS
a During lead feeding			
		mgm	*mgm*
149	1	0	5 91
	2	0	23 70
	3	0 34	36 07
	4	0 22	17 70
	5	0 76	27 90
179	1	0	63 77
	2	0	48 84
148	1	0	170 00
b After subcutaneous administration of lead			
292	1	0	3 13
	2	0 23	0 15
296	1	0 48	0 31
	2	0 56	1 06
286	1	0 36	0 30
	2	0 42	0 16

remainder had merely passed unchanged through the gastro-intestinal tract Moreover, much of the lead absorbed was probably removed from the portal blood by the liver and excreted without ever reaching the general circulation Thus, lead in the feces following the ingestion of lead compounds is no real index of the amount of lead actually entering the organism, but the small amounts of lead occasionally found in the urine indicate that lead is being transported in the blood

stream When all the lead absorbed enters the general circulation directly—a condition which can be produced experimentally by subcutaneous injection of lead—excretion is much more gradual Several factors explain this difference (*a*) The fact that unabsorbed lead cannot be excreted from a subcutaneous deposit, (*b*) the slower entrance of lead into the blood stream, and (*c*) the tendency of the

TABLE 22

Comparative rates of excretion of lead in urine and feces of man

PATIENT	NUMBER OF CONSECUTIVE THREE DAY PERIODS INCLUDED IN AVERAGE	AVERAGE EXCRETION PER PERIOD		RATIO Urine Feces
		Urine	Feces	
		mgm	*mgm*	
J S	5	0 23	0 39	1 1 7
J S	2	0 43	1 15	1 2 7
R D W	3	0 28	1 10	1 4 0
J L	1	0 78	0 96	1 1 2
J L	1	0 26	0 42	1 1 6
D M	4	0 46	0 97	1 2 1
D M	4	0 29	0 85	1 3 0
P M	2	0 15	0 06	1 0 4
P M	2	0 45	0 17	1 0 4
P M	4	0 30	0 78	1 2 6
P M	4	0,25	1 04	1 4 0
P M	3	0 26	0 98	1 3 8
J T	2	0 72	1 56	1 2 1
J T	2	0 58	1 86	1 3 2
J T	4	0 38	0 29	1 0 8
J T	4	0 23	1 11	1 4 8
M M	1	0 12	0 51	1 4 0
Average				1 2 5

skeleton to remove and retain circulating lead Although there is not only an absolute but also a relative decrease in the quantity of lead excreted in the feces after subcutaneous injection, the rate of excretion by this route is generally greater than that by the kidney

To furnish comparative data on elimination of lead in urine and feces, observations made on patients with chronic plumbism proved more satisfactory than those on animals The subjects were under observation in a hospital and analyses were not made until they had been removed from exposure to lead for some time For chemical analysis,

the urine and feces were collected separately in three-day periods marked by the administration of carmine The results presented in table 22 show that there is no definite quantitative ratio between urinary and fecal excretion Much more lead is eliminated by the gastro-intestinal tract than by the kidney, although in six of the periods included in the averages shown in table 22, small amounts of lead appeared in the urine when none could be detected in the feces This is unusual, however, and in these observations the average ratio between the amounts excreted in urine and feces is 1 2 5 More striking and significant, however, is the regularity of the urinary excretion, which never becomes great yet was negative in only 5 of the 48 periods studied Thus the extreme ranges of urinary excretion for three-day periods in all the cases studied were from 0 to 0 96 mgm of Pb, while the corresponding elimination in the feces varied from 0 to 2 05 mgm The averages as presented in table 22 naturally vary much less—from 0 12 to 0 78 mgm of Pb in urine, and from 0 06 to 1 86 mgm in feces Obviously the excretion of large amounts of lead must be accomplished by the gastro-intestinal tract, and therefore the greater the total output of lead the greater becomes the disproportion between the quantities of lead eliminated in urine and feces

This simple observation that the amount of lead which the kidney can excrete is distinctly small, while that which the gastro-intestinal tract can eliminate is unlimited, probably depends upon very fundamental factors of excretion An analogy certainly seems to exist between the excretion of lead and calcium It has frequently been observed that a high phosphate diet tends to increase the proportion of calcium excreted in the feces, while the ingestion of hydrochloric acid, on the contrary, increases the urinary output Although the reasons for this have not been clearly worked out, a possible explanation may be found in the relative solubility of the compounds formed Thus calcium chloride, a soluble salt, appears in the urine, while calcium phosphate, which is very insoluble, is excreted almost entirely by the intestine As has been shown in section IV, lead exists and is transported in the organism chiefly in the form of highly dispersed colloidal lead phosphate, which is very insoluble The amounts of this which may be dissolved are small and vary with the hydrogen ion

concentration in the organism The fact that the acidity is always very low may possibly explain the limited excretion of lead in urine

Excretion of lead by the skin The observation that the skin of patients suffering from plumbism can be darkened by exposure to hydrogen sulphide gas has been considered by some investigators as evidence of cutaneous absorption of lead, and by others as proof of cutaneous excretion Du Moulin's early work was verified by Lavrand (248) who carried out extensive studies showing that much of the coloration produced by exposure to hydrogen sulphide is due to the formation of *iron* and not *lead* sulphide Furthermore, Lavrand found that as time elapsed after exposure, the degree of coloration gradually became less—an indication that the lead present soon after exposure is merely contamination and has not been excreted The same criticism may probably also be applied to Oliver's (337, page 115) observation that lead can be detected in perspiration, and likewise to the findings which led Meillere to believe that lead is excreted not *by* but *to* the skin, hair, and other keratinous tissue where it is stored in some harmless chemical combination (292) The figures already presented in chapter VI which show the absence of any accumulation of lead in the hair or skin are evidence against this idea of an "internal excretion" of lead to the keratinous tissue

Summary The more important points regarding the excretion of lead are that

Lead is excreted by the entire gastro-intestinal tract, in the bile, by the kidney, and possibly also to a slight extent by the skin

By far the greatest proportion of lead is excreted in the feces, though small amounts are usually eliminated simultaneously by the kidney

The small amounts of lead in urine are of much greater clinical and diagnostic significance, for if lead is being ingested it is impossible to determine whether the lead in the feces has been absorbed or has merely passed unchanged through the alimentary canal

VIII FACTORS INFLUENCING THE RATE OF LEAD EXCRETION IN ANIMALS

After absorption has ceased, the rate at which lead can be excreted from the organism depends chiefly upon the stability of the skeletal store Since both lead and calcium are held in the body at a

common site and the chemical behavior of some of their salts *in vitro* is very similar, it seems quite probable that the same physiological conditions might favor the liberation or retention of both A review of information regarding the chief factors which influence calcium metabolism furnishes many suggestions about a possible control of lead storage

Calcium excretion Many different physiological and pathological factors influence the calcium economy of the organism Most obvious of these in determining whether calcium shall be drawn from or added to the skeletal store is diet Not only the relation of the actual calcium intake (420) (346) (112) (160) (169) (354) to the requirements of the body, but also the vitamine content (202) (426) (423) (424) (211) (170) (220) and the acid or basic nature of the food (41) (33) (161) (134) play an important part Perhaps even more potent and fundamental changes are produced within the organism by such conditions as (*a*) acidosis, whether due to excessive fatigue, incomplete oxidation of inorganic acids, or acute infection (436) (397) (186) (329) (498), (*b*) pregnancy and the fetal demands upon the calcium reserve (436) (42) (97) (98), (*c*) lactation, which involves a prolonged loss of calcium (146) (150) (291) (436) (97), and (*d*) variations of endocrine activity (27) (180) (44) Medication also has a profound influence upon calcium metabolism, and since the changes which it produces may be experimentally controlled, it provides a practical means of studying the stability of the lead stored in the organism The types of such medication which have been most studied fall roughly into groups (*a*) calcium salts, (*b*) antagonistic cations such as magnesium, potassium, and sodium, and (*c*) acids and alkalies In addition to these, sunlight has been found to produce marked effects

The administration of calcium salts does not invariably change the calcium balance toward the positive side as might be expected Berg (33) found that ingested calcium phosphate adds nothing to the calcium store since it is merely excreted unchanged in the feces, and this is in accord with the observations of Étienne (123) and Bonnamour, Sarvonat *et al* (44), who showed that prolonged administration of calcium chloride causes marked decalcification following a slight transient retention This drain upon skeletal calcium was shown by Étienne to be so severe that deformities resembling osteomalacia developed in his experimental animals Berg believed that any inorganic anion aids such depletion of the calcium store and advised administration of calcium lactate or bicarbonate to induce a retention Steenbock, Hart *et al* (437), however, found that the addition

of calcium lactate, carbonate, chloride, silicate or phosphate to diets adequate in all respects except for their calcium content, maintained normal growth in rats The decalcifying effect of magnesium, sodium, and potassium salts depends upon the fact that the organism tends to maintain a normal ratio between the concentrations of these various salts in the body fluids Thus, if one is present in excess, the ratio is restored by calling upon the reserves of the others Among the salts which have been used to produce decalcification in this way are sodium chloride (333) (207), potassium iodide (134), magnesium chloride and citrate (41), and magnesium oxalate (117) As Berg suggested, part of the efficiency of some of these may be due to the action of inorganic anions, but the antagonistic cations also seem to play a part.

The chemical reasons for the administration of acid to liberate stored calcium are obvious The solvent action of many acids has been tested and the most effective have proved to be those which are non-toxic and cannot be readily burned in the organism but must be neutralized by the bases present Of the mineral acids, hydrochloric (438) (159) (163) (174) and phosphoric (ingested either as such or as the acid phosphate) produce marked decalcification (33) Organic acids are much less useful Sarvonat and Roubier (395) experimented upon animals with oxalic acid and others have frequently used the less toxic and much less effective lactic and citric acids (134) That administration of alkalies would produce an effect quite contrary to that of acids, i e , would favor retention of calcium, is an apparently reasonable assumption Introduction of an alkali in the organism, of course, helps neutralize any acid present and so decreases the demand on the basic reserves But experiments demonstrate that when an excess of alkali in the form of sodium bicarbonate is introduced into a normal individual, there is a tendency toward calcium loss rather than retention (159) (396) The effect of sunlight on calcium metabolism is not understood but it is known that exposure to the sun's rays favors storage of calcium in the bones of children Sunlight is therefore extensively used as a therapeutic agent in rickets (212) (425)

Lead excretion This brief survey suggests several logical methods of investigating the factors which control the store of lead within the skeleton The most practical of these involve variations of diet and medication with acids and salts which liberate reserve stores of calcium They should theoretically also prove effective in mobilizing lead which has been deposited in the bones Because of the warning frequently repeated in the literature that administration

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f potassium iodide may suddenly liberate stored lead and cause evere intoxication, it has seemed best to carry out experiments ith animals before attempting to treat patients with these more owerful decalcifying agents

In these experiments, cats which had been severely poisoned either by rolonged lead feeding or by the insufflation of lead carbonate, were allowed

TABLE 23

Effect of starvation and hydrochloric acid on lead excretion

NUMBER OF CAT	TREATMENT	WEEKLY DOSE OF MOLAR SOLUTION	CALCIUM INTAKE	WEEKLY EXCRETION OF LEAD	BLOOD CO_2	BRIEF HISTORY
		cc.		*mgm*	*volume per cent*	
15	Control	0	High	3 64		Received 3 32 grams of lead in 32 doses by mouth from March 9, to May 22, 1922 This observation started November 1, 1922
	Control	0	High	4 69		
	HCl and starvation	43	0	2 24	25 2	
	Control	0	High	2 45		
	Control	0	High	0		
11	Control	0	High	0		Received 15 61 grams of lead in 54 doses by mouth from January 20, to June 8, 1922 This observation started October 1, 1922
	Control	0	High	0		
	HCl and starvation	49	0	8 19	20 7	
	Control	0	High	0*		
	Control	0	High	6 44		
8	Control	0	High	1 48		Received 11 22 grams of lead in 68 doses by mouth from January 20, to June 8, 1922 This observation started September 1, 1922
	Control	0	High	4 27		
	HCl and starvation	52 5	0	6 02	23 6	
	Control	0	High	5 29		
	Control	0	High	0		
	HCl and starvation	42	0	3 99	22 3	
	Control	0	High	0		
	Control	0	High	0 21		

* Very constipated

to recover completely after the administration of lead had ceased They were then confined in metabolism cages so that their excreta could be analyzed during control and medication periods As no attempt was made to separate urine from feces, the figures presented here represent the combined excretion by the two routes. In one series of experiments the animals were

TABLE 24

Effect of ammonium chloride on lead excretion

CAT		PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5	PERIOD 6	PERIOD 7	REMARKS
217	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by lung January 26, 1923, February 2, 1923, February 12, 1923 This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	149 5	112 1	130 8	0	0	
	Pb excreted weekly mgm	1 22	0	0	1 27	0	0 17	1 83	
337	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by mouth October 5, 1922, to March 1, 1923 This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	149 5	112 1	130 8	0	0	
	Pb excreted weekly mgm	0	1 07	0 28	1 16	2 88	0	0 25	
305	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by lung June 29, 1923 This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	130 8	112 1	140 0	0	0	
	Pb excreted weekly, mgm	0	0 24	0 13	2 30	0 74	0 53	2 10	
308	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by lung June 29, 1923 This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	149 5	112 1	140 0	0	0	
	Pb excreted weekly, mgm	0	0 29	0	1 11	0 13	0 29	0	
303	Calcium diet	High	High	Low	Low	Low	High	High	Leaded by lung June 29, 1923 This observation started December 23, 1923
	M/1 solution weekly, cc	0	0	149 5	112 1	149 5	0	0	
	Pb excreted weekly, mgm	0	0	0	1 04	2 77	1 14	0 60	

at first starved during the administration of acid in order to increase the drain upon the calcium reserve by the production of acid within the organism Later, when longer observation periods were desirable, they received a diet consisting of meat or liver which contain a very little calcium The nutrition was adequate except for calcium

The data obtained after administration of HCl demonstrate that the acid caused a prompt increase in excretion (see table 23) The

TABLE 25

The effect of ammonium chloride on lead excretion

CONTROL ANIMALS			TREATED ANIMALS		
Number of period	Weekly excretion of lead		Number of period	Weekly excretion of lead	
	Cat 284	Cat 238		Cat 478	Cat 495
	mgm	*mgm*		*mgm*	*mgm*
1	0	11 23	1	5 57	4 90
2	2 87	3 31	2	1 96	4 69
3	2 17	1 91	3	4 47	1 84
4	0	0	4	3 52	3 44
5	0	0	5	2 26	0 12
6	0 17	0	6	3 17	3 50
7	0 17	0	7	4 28	2 40
8	0 73	6 63*	8	5 08	5 07
9	0	0 25	9	4 92	7 70
Average	0 68	2 59	Average	3 91	3 74
Average for two controls		1 63	Average for two treated animals		3 82

* Animal sick with snuffles

low capacity of blood plasma for CO_2 is evidence of a considerable degree of acidosis Although acid may be thus ingested in effective doses (in cats by stomach tube) it frequently causes nausea and vomiting, and large doses cannot be taken without gastric disturbance

J B S Haldane (183) has recently reported that rather large doses of ammonium chloride cause marked acidosis in man without digestive disturbances Since this salt liberates one molecule of hydrochloric acid when 2 molecules of ammonia combine with carbon dioxide to form urea in the body, a means is provided of administering hydrochloric acid indirectly Although in very large doses

TABLE 26

Effect of phosphoric acid on lead excretion

	TREATMENT	WEEKLY DOSE OF MOLAR SOLUTION	CALCIUM INTAKE	WEEKLY EXCRETION OF LEAD	BRIEF HISTORY
				Cat 217	
		cc		*mgm*	
	Control	0	High	0	Received ±2 cc of lead carbonate suspension by lung on January 26, February 2, and February 12, 1923
	Control	0	Low	0 12	
A	H_3PO_4	32 0	Low	0 27	
	H_3PO_4	38 2	Low	0 40	Observation A started May 17, 1923
	Control	0	High	0 21	
	Control	0	High	0 72	Observation B started April 30, 1924
	Control	0	High	3 22	
B	H_3PO_4	82 7	Low	4 78	
	H_3PO_4	63 6	Low	3 67	
	Control	0	High	1 47	
	Control	0	High	1 69	
				Cat 337	
	Control	0	High	0	Received 7 7 grams of lead as lead acetate solution by mouth from October 5, 1922, to March 1, 1923
	Control	0	Low	0 16	
A	H_3PO_4	25 4	Low	0 50	
	H_3PO_4	38 3	Low	1 22	Observation A started May 17, 1923
	Control	0	High	1 44	
	Control	0	High	1 56	Observation B started March 23, 1924
	Control	0	High	0 69	
B	H_3PO_4	82 7	Low	4 12	
	H_3PO_4	63 6	Low	3 01	
	Control	0	High	0 24	
				Cat 305	
	Control	0	Low	0 25	Received ±2 cc of lead carbonate suspension by lung June 29, 1923
	Control	0	High	0	
A	H_3PO_4	93 8	Low	0	
	H_3PO_4	66 1	Low	1 26	
	Control	0	High	0	Observations A and B are continuous and started September 2, 1923
	Control	0	High	0	
B	H_3PO_4	76 5	Low	1 36	
	H_3PO_4	114 7	Low	1 70	
	Control	0	Low	0	
	Control	0	High	0 50	

TABLE 27

Effect of di-ammonium phosphate on lead excretion

CAT		PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5	PERIOD 6	REMARKS
238	Calcium diet	High	Low	Low	Low	High	Low	Leaded by lung November 5, 1923 This observation started January 4, 1924
	M/1 solution weekly, cc	0	45 4	49 2	68.2	0	0	
	Pb excreted weekly, mgm	0 25	4 92	3 71	1 57	1 57	0 39	
284	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung November 5, 1923 This observation started December 28, 1923
	M/1 solution weekly, cc	0	0	45 4	49 2	68 2	0	
	Pb excreted weekly, mgm	0 73	0	0 29	0 19	0	0 40	
600	Calcium diet	High	Low	Low	Low	High	High	Leaded by lung November 5, 1923 This observation started December 28, 1923
	M/1 solution weekly, cc	0	0	49 2	68 2	0	0	
	Pb excreted weekly, mgm	0 48	1 35	0 27	1 99	0 38	0 87	

it is an emetic and the amount which can be administered to animals is therefore somewhat limited, doses which can be tolerated increase the excretion of lead when the diet contains little calcium (table 24)

Other experiments were also performed to demonstrate the effect of ammonium chloride (112 cc M/1 solution per week) Four animals were given lead intratracheally, and while absorption was still going on, two of them were treated with daily doses of ammonium chloride The animals receiving ammonium chloride excreted approximately twice as much lead as did the controls and provide an excellent illustration of the action of this drug in preventing complete storage of lead in the bones (see table 25)

Phosphoric acid is less highly dissociated than hydrochloric and consequently can be tolerated in higher concentration It causes vomiting less frequently and increases the rate of elimination of lead in cats very promptly (table 26) A few experiments performed to test the acid-forming effect of di-ammonium phosphate demonstrated that doses which can be tolerated produce rather variable changes Although the figures (see table 27) show that the excretion of lead increased slightly, this salt was quite ineffective in two of the three cases studied Later work with man has confirmed this observation

Since tartaric acid is a well known solvent for lead phosphate *in vitro*, it also was administered to our animals It proved toxic, however, especially to the kidney, and did not favor elimination of lead to any considerable degree

Observations on the effect of sodium bicarbonate have proved most instructive in pointing out the essential mechanism of the action of acids and alkalies upon stored lead When administered to a starving animal with acidosis sufficient to mobilize the stored lead, sodium bicarbonate favors storage of lead (310) probably by restoring the normal hydrogen ion concentration of the organism But when given in excessive doses to an animal normal except for a deposit of lead in the skeleton, sodium bicarbonate tends to increase greatly the elimination of lead (see table 28) The chemical explanation of these apparently opposite effects is suggested by the work of Fairhall and Shaw (129) (see section IV) Their work suggests that while lead phosphate is least soluble within the usual range of hydrogen ion concentrations in the organism, a relatively slight increase of either acidity or alkalinity promptly increases its solu-

TABLE 28

Effect of sodium bicarbonate on the excretion of lead

CAT		PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5	PERIOD 6	REMARKS
238	Calcium diet	High	Low	Low	Low	Low	High	Leaded by lung November 5, 1923. This observation started February 1, 1924
	M/1 solution weekly, cc	0	0	119	119	0	0	
	Pb excreted weekly, mgm	1 57	0 39	2 63	1 41	1 93	0 75	
284	Calcium diet	High	Low	Low	Low	Low	High	Leaded by lung November 5, 1923 This observation started February 1, 1924
	M/1 solution weekly, cc	0	0	119	119	0	0	
	Pb excreted weekly, mgm	0 40	3.03	1 86	2 13	2 60	0	
600	Calcium diet	High	Low	Low	Low	Low	High	Leaded by lung November 5, 1923. This observation started February 1, 1924
	M/1 solution weekly, cc	0	0	119	119	0	0	
	Pb excreted weekly, mgm	0 38	0 87	4 69	2 18	0 21	0 33	
337	Calcium diet	High	High	Low	Low	Low	High	Leaded by mouth Total 7 7 grams Last dose March 11, 1923 This observation started January 27, 1924
	M/1 solution weekly, cc	0	0	142 8	95 2	0	0	
	Pb excreted weekly, mgm	0	0 25	0	1 32	1 24	2 57	
217	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung January 26, 1923, February 2, 1923, February 12, 1923 This observation started January 27, 1924
	M/1 solution weekly, cc	0	0	142 8	95 2	0	0	
	Pb excreted weekly, mgm	0 17	1 83	1 38	5 77	5 07	4 13	
303	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung June 29, 1923. This observation started January 27, 1924
	M/1 solution weekly, cc	0	0	142 8	95 2	0	0	
	Pb excreted weekly, mgm	1 14	0 60	2 36	4 11	3 17	1 83	
305	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung June 29, 1923. This observation started January 27, 1924
	M/1 solution weekly, cc	0	0	142 8	95 2	0	0	
	Pb excreted weekly, mgm	0 53	2 60	0	1 78	2 35	0 22	
308	Calcium diet	High	High	Low	Low	Low	High	Leaded by lung June 29, 1923. This observation started January 27, 1924
	M/1 solution weekly, cc	0	0	142 8	95 2	0	0	
	Pb excreted weekly, mgm	0 29	0	6 25	6 18	5 78	0 73	

bility It seems possible therefore that acids and alkalies liberate lead by shifting the hydrogen ion concentration, perhaps only locally, to a point sufficiently acid or alkaline to increase the solubility and permit liberation of some of the stored lead phosphate

Too few animals have been studied and too many complicating factors are involved to warrant any conclusions as to the relative efficiency of the different forms of medication in ridding the organism of lead, but the data do show that decalcifying agents tend to set free lead without—in so far as can be judged from animal experiments—producing any alarming symptoms of lead intoxication The slight loss of appetite and general malaise which appear during this forced treatment promptly disappeared as soon as medication ceased Among the animals to which acid was administered while lead was being actively absorbed from the lungs, there were a few exceptions to this The acid seemed to hasten absorption as well as to liberate stored lead If medication was continued under these conditions the poisoning sometimes became fatal This corresponds to the results obtained by Melsens (298) and others when poisoned animals were treated with potassium iodide The appearance of a lead line, marked loss of appetite, increasing weakness and constipation, however, usually gave ample warning that the organism was being overwhelmed by lead, and symptoms quickly disappeared if medication was stopped and the calcium intake increased From the point of view of safety, therefore, the use of these decalcifying agents to increase the elimination of lead in man seemed justified

To determine how completely retained lead can be removed from the organism by reasonably prolonged medication, the tissues of these poisoned animals were analyzed for lead after treatment The data from thirteen cats are presented in table 29 Comparatively large amounts had been eliminated by these cats For example, in the periods determined for cat 217, 91 mgm of lead had been excreted over a period of four hundred and twenty days of observation, and for cat 337, after lead by mouth, 51 mgm in three hundred and fifty days However, considerable quantities were still stored in most cases after prolonged periods of observation The detection of unabsorbed lead in the lungs of the cats which had received lead by insufflation of course indicates that continued absorption may have

TABLE 29

Analysis of tissues of cats after prolonged medication

NUMBER OF CAT	METHOD OF LEAD ADMINISTRATION	APPROXIMATE AMOUNT OF LEAD GIVEN	INTERVAL BETWEEN DOSE AND DEATH	TOTAL LEAD IN BODY*	PER CENT OF TOTAL IN SKELETON	UNABSORBED LEAD STILL IN LUNG	NUMBER OF MEDICATION PERIODS	MEDICATION RECEIVED
		mgm	*days*	*mgm*		*mgm*		
8	By mouth	11,200	244	23 33	97 3		3	HCl (2) Lactic and phosphoric acid (1)
11	By mouth	15,610	172	108 86	98 5		2	HCl (2)
15	By mouth	3,320	307	49 82	99 3		4	HCl (1), lactic acid (2) H_3PO_4 (1)
337	By mouth	7,700	506	35 28	96 8		19	Tartaric acid (2), H_3PO_4 (6) NH_4Cl (7), thyroid extract (2), $NaHCO_3$ (2)
217	By lung	600–700	532	71 14	100 0	3 78	21	KCl (2), KI (2), lactic (2) H_3PO_4 (8), NH_4Cl (5), $NaHCO_3$ (2)
305	By lung	250	390	9 19	100 0	0 56	11	H_3PO_4 (6), NH_4Cl (3) $NaHCO_3$ (2)
306	By lung	250	173	38 37	97 7	7 37	8	H_3PO_4 (6), NH_4Cl (2)
308	By lung	250	387	21 40	100 0	3 20	13	HCl (2), lactic (2), HCl (2), NH_4Cl (3) $NaHCO_3$ (2), H_3PO_4 (2)

238	By lung	250	259	35 41	100 0	5 35	7	$(NH_4)_2HPO_4$ (3), $NaHCO_3$ (2) H_3PO_4 (2)
284	By lung	250	265	10 13	100 0	4 88	7	$(NH_4)_2HPO_4$ (3), $NaHCO_3$ (2) H_3PO_4 (2)
600	By lung	250	173	25 52	96 5	11 50	6	$(NH_4)_2HPO_4$ (2), $NaHCO_3$ (2), H_3PO_4 (2)
478	By lung	300	197	30 71	100 0	9 00	8	NH_4Cl (8)
495	By lung	300	197	12 84	100 0	0	8	NH_4Cl (8)

* Exclusive of lung in cases receiving lead by insufflation

been partly responsible for the lead remaining in the tissues despite medication The quantity of retained lead in these animals, however, is not greater than in those receiving lead by mouth In the latter, absorption must have ceased within a few days after the last dose was given While these results on the whole discourage the hope of completely freeing the organism from lead, experiments on man in which subjective symptoms can be accurately determined alone can decide the relative desirability of (*a*) favoring complete storage of lead in the bones by high calcium intake and a careful maintenance of a normal acid-base equilibrium, or (*b*) eliminating as much lead as possible by medication before establishing these measures to insure storage of the remaining lead

Summary. It has been shown by chemical studies with animals that an analogy exists in the metabolism of calcium and lead Various decalcifying agents have been shown also to increase the lead output Conversely, conditions favoring calcium retention also tend toward a complete storage of lead in the bones

Although the lead elimination had been considerably increased, the analysis of the tissues of animals after prolonged medication showed significant amounts of lead still retained by the bones While it is probably impossible to "de-lead" a poisoned individual completely, further experiments on man alone can demonstrate the relative desirability of a preliminary medication to increase the excretion of lead or of working from the first for a complete storage of lead in the skeleton

IX. FACTORS INFLUENCING THE RATE OF LEAD EXCRETION IN MAN

The experiments with animals have indicated that the factors determining excretion of lead from the organism are practically the same as those determining liberation of lead from the bones As lead is stored in the hard bone in man just as in animals (309), we therefore have employed upon patients suffering from lead poisoning the same methods which apparently proved effective in liberating stored lead in cats The determination of the amount of lead eliminated in the excreta is, of course, but an indirect and inexact measurement of the quantity liberated from the bones, for lead may circulate through the body and be re-deposited without appearing

in either urine or feces But the amount thus forming the "lead stream" in the blood is too minute to measure by any quantitative chemical methods now available

In a special ward of three beds at the Massachusetts General Hospital, our patients have been under the charge of a special metabolism nurse who had no duties except to see that the necessary regime was carried out Patients were usually transferred from the regular wards after the definite diagnosis of relatively severe lead poisoning had been established, and were promptly started on their special regime All the excreta were collected, the urine in twenty-four-hour specimens (preserved by powdered thymol), the feces in individual specimens Since chemical analyses were made of the excreta for three day periods, 0 3 gram of carmine alum lake was administered orally every third day to permit accurate collection of feces as well as urine This dye was given at 1 P M and its first appearance in the feces marked the end of the period, the collection of urine for this same period ended at 6 P M

In order to do away with as many variable factors as possible during medication, the patients were given the same low calcium diet daily During the first few days they were allowed to choose whatever seemed most attractive from the list of foods containing little or no calcium, and thereafter the daily ration was kept strictly uniform The patients accepted this monotonous diet well All food was weighed, and if any was not eaten its quantity was carefully determined and deducted from the total A list of the different foods given and their calcium content follows

Foods allowed in low calcium diets

	Content of calcium in per cent
Milk free bread	0 011
Regular bread	0 041
Round steak	0 008
Liver	0 006
Chicken	0 011
Ham	0 022
Potato (raw)	0 011*
Macaroni	0 018
Rice (uncooked)	0 007
Rice (boiled)	0 006
Canned tomatoes	0 005
Canned corn	0 005
Apple, fresh	0 010*
Apple, dried	0 021

Peaches, dried	0 026
Bananas, fresh	0 007*
Butter fat	
Salt, pepper, sugar	
Coffee, tea	

* From Sherman

The calcium intake was raised either by the addition of calcium lactate and milk to this uniform diet or by allowing the patient to receive the regular hospital diet plus a quart of milk and two grams of calcium lactate per day.

Under such uniform conditions, the factors influencing the excretion of lead, when absorption is no longer active, may be studied with comparative ease Thus, sixty-five satisfactory observations (with an average duration of more than three weeks) have been made of the effects of various medications which influence the balance of salts, the calcium balance, or the acid-base equilibrium, or produce catharsis Each new procedure was started with great caution in order to avoid precipitation of any such acute manifestation as colic. The influence of (*a*) magnesium sulphate, (*b*) potassium iodide, (*c*) potassium chloride, (*d*) high calcium intake, (*e*) low calcium intake, (*f*) diammonium acid phosphate, (*g*) phosphoric acid with both high and low calcium diet, (*h*) hydrochloric acid with high and low calcium diet, (*i*) ammonium chloride, (*j*) sodium bicarbonate, and (*k*) sodium citrate has been studied in twenty-seven of our cases—for the most part within the last two years Symptoms of intoxication by lead appeared rarely during treatment, and at the time of discharge from the hospital all the patients had improved markedly—they had gained weight and were relieved of active symptoms In three of the many patients treated a mild colic developed and in one encephalopathy recurred after administration of ammonium chloride, but this promptly disappeared with the restoration of a positive calcium balance.

As space does not permit a detailed outline of all the observations, some will merely be mentioned in a general way and the others will be reported in the summaries of the effects of medication

Although before and after nearly every period of medication control observations were made, these could not always continue as long as was

TABLE 30

Control periods consecutive observations of three day periods without medication other than change in diet

DATE	DIET	LEAD EXCRETION			CALCIUM	
		Urine	Feces	Total	Intake	Output
	P M					
		mgm	*mgm*	*mgm*	*grams*	*grams*
April 7 to April 28	Low calcium plus calcium lactate	0 52	0 25	0 77	2 172	2 350
	Low calcium plus calcium lactate	0	0 87	0 87	2 172	1 617
	Low calcium plus calcium lactate	0 44	0	0 44	2 172	2 661
	House diet	0 34	0 06	0 40		
	House diet	0 24	0 67	0 91		
	House diet	0 26	0 76	1 02		
	House diet	0 33	0 75	1 08		
May 19 to June 2	Low calcium diet	0 33	0 47	0 80	0 360	0 622
	Low calcium diet	0 33	1 22	1 55	0 408	0 711
	Low calcium diet	0 41	0 56	0 96	0 411	0 682
	Low calcium diet	0	0 80	0 80	0 411	1 450?
	Low calcium diet	0 26	1 57	1 83	0 411	0 868
June 24 to July 11	Low calcium diet	0 47	0 61	1 08	0 357	0 991
	Low calcium diet	0 46	1 30	1 76	0 357	0 707
	Low calcium then house diets	0 22	0 96	1 18		2 221
	House diet	0 10	0 69	0 79		4 467
	House diet	0 28	1 10	1 38		3 952
	House diet	0	0	0		3 282
	D J M					
May 3 to May 18	House diet	0 43	0 52	0 95		4 500±
	House diet	0 72	0 33	1 05		2 600
	Low calcium diet	0 44	1 12+	1 56+	0 341	
	Low calcium diet	0	1 80	1 80	0 355	0 609
	Low calcium diet	0 43	0 74	1 17	0 355	0 756
June 3 to June 18	Low calcium diet after $NaHCO_3$	0 48	1 09	1 57	0 420	0 950
	Low calcium diet after $NaHCO_3$	0 26	1 30	1 56	0 420	0 937
	Low calcium diet after $NaHCO_3$	0 35	0 91	1 26	0 411	0 692
	Low calcium diet after $NaHCO_3$	0 35	1 21	1 66	0 405	0 897
	Low calcium diet after $NaHCO_3$	0 20	0	0 20	0 348	0 815
July 18 to August 4	House diet	0 69	0	0 69		2 249
	House diet	0	0	0		2 839
	House diet	0 22	0	0 22		
	House diet	0	0	0		
	House diet	0	0	0		
	House diet	0	0	0		

TABLE 30—*Continued*

DATE	DIET	LEAD EXCRETION			CALCIUM	
		Urine	Feces	Total	Intake	Output
	J T					
		mgm	*mgm*	*mgm*	*grams*	*grams*
April 7 to April 27	Weighed diet plus calcium lactate	0 39	0	0 39	2 172	2 720
	Weighed diet plus calcium lactate	0	0	0	2 172	2 765
	Weighed diet plus calcium lactate	0 32	1 43	1 75	2 172	3 118
	House diet	0 33	0 97	1 30		
	House diet	0 21	0	0 21		
	House diet	0 31	0 47	0 78		
	House diet	0 43	0 40	0 83		
May 19 to June 2	Low calcium diet after $NaHCO_3$	0	1 94	1 94	0 357	0 685
	Low calcium diet after $NaHCO_3$	0	1 38	1 38	0 405	0 731
	Low calcium diet after $NaHCO_3$	0 49	1 76	2 25	0 411	0 865
	Low calcium diet after $NaHCO_3$	0 43	0	0 43	0 411	
	Low calcium diet after $NaHCO_3$	0	1 30	1 30	0 411	1 109
June 27 to July 18	Low calcium diet	0 45	1 34	1 79	0 357	0 880
	Low calcium diet for two days	0 25	2 04	2 29		4 659
	House diet	0 31	0	0 31		10 998
	House diet	0 18	0	0 18		8 567
	House diet	0 13	0	0 13		9 045
	Low calcium diet	0 42	1 01	1 43	0 316	1 519
	Low calcium diet	0	2 34	2 34	0 399	1 566
	M R					
July 3 to July 17	Milk, during severe lead colic	0 74	2 83	3 57		
	Milk	0	0	0		
	Milk	0 56	0 59	1 15		2 033
	Low calcium diet	0 22	0	0 22	0 252	0 794
	Low calcium diet	0	0 72	0 72	0 322	0 793
July 30 to August 10	House diet	0	0	0		
	House diet	0	0	0		
	House diet	0	0 37	0 37		
	House diet	0 09	0 79	0 88		

longed time In several patients, however, it was possible to make a series of such observations which is of value as a control (table 30) These demonstrated considerable variation in which both diet and constipation played some part As shown in animals, a diet deficient in calcium favors excretion, and this may also be seen in the three observations on the patient J T, where the third period (June 27 to July 18) particularly shows a striking effect of change of diet There is a possibility that the longer the deficiency in calcium the greater the effect of medication on the excretion of lead Consequently several cases are reported in full (figs 20, 21, and 22) to show the course of prolonged observations

Individual variations in the control periods necessitate merely qualitative interpretation of the results of medication As the number of observations is too small to permit statistical or mathematical study, and as diet and individual variations complicate interpretation greatly, only average figures will be given here as evidence of the effects of the different medications Detailed data are reported in a separate paper (19)

Factors influencing excretion of lead Administration of cathartics has long been the favorite treatment of lead intoxication Croton oil, which was recommended by Tanquerel, has now been largely replaced by magnesium sulphate The exact effect of these cathartics upon the rate of excretion of lead has not been previously determined In table 31 are data obtained from three observations after daily administration of magnesium sulphate (in one case one-third ounce per day, in two cases one ounce per day) The average excretion of lead in nine three-day periods during ingestion of magnesium sulphate was 0 53 mgm , that in five control periods was 0 30 mgm Thus, although it has a marked cathartic action, magnesium sulphate does not greatly influence the total lead excretion It does, however, cause improvement in cases of lead colic, probably by relieving constipation and cleansing the gastro-intestinal tract, as well as by relieving spasm of smooth muscle, and not by increasing the rate of elimination of lead

Potassium iodide Quite another type of medication which has been employed for many years is the administration of potassium iodide Melsens (297), who recommended its use in 1840, carried out experiments on dogs which have been supplemented by further

investigations by Parkes (352), Pouchet (366), and Dixon Mann (279) (see page 210) Their results are contradictory Parkes and Pouchet believed that potassium iodide increased excretion, but Mann could not observe that it had any such effect As no definite conclusion had been drawn when our investigation was started, one of the first problems was to determine its efficiency Analysis of the

TABLE 31

The influence of medication

The average total excretion of lead for three-day periods

MEDICATION	NUMBER OF CASES	CONTROL PERIODS		PERIODS DURING MEDICATION		FIRST PERIOD AFTER MEDICATION		SUBSEQUENT CONTROLS		RATIO: AVERAGE VALUE DURING TREATMENT / AVERAGE VALUE BEFORE TREATMENT
		Number of periods	Lead excreted	Number of periods	Lead excreted	Number of periods	Lead excreted	Number of periods	Lead excreted	
			mgm		*mgm*		*mgm*		*mgm*	
Phosphoric acid with low calcium diet	10	19	0 81	45	2 51	9	0 92	15	0 53	3 1
Phosphoric acid with normal diet	12	22	0 46	43	1 10	7	1 54*	2	0 42	2 4
Hydrochloric acid	4	7	0 31	13	0 88	2	0 42	1	0	2 8
Ammonium phosphate	2	5	1 27	11	1 60	1	0 36	1	0 95	
Ammonium chloride	8	17	0 80	41	2 35	6	1 17	14	0 90	2 9
Tartaric acid	2	1	0 63	7	0 52	2	0 69	1	1 37	
Lactic acid	2	4	0 60	5	0 54					
Sodium bicarbonate	7	17	0 71	34	1 93	5	1 55	15	1 15	2 7
Sodium citrate	1	2	1 51	5	1 72					
Potassium iodide	9	10	0 68	28	1 29	6	1 19	8	0 50	1 9
Ammonium iodide	1	2	0 87	4	2 34					
Potassium chloride	2	2	0 57	7	0 57	1	0 53			
Magnesium sulphate	3	3	0 41	9	0 53	2	0 14			

* Greatly elevated by one high figure of 5 69 mgm

excreta both before and during the use of potassium iodide clearly demonstrated (see table 31 and fig 20) that elimination of lead increases during ingestion of this drug When no lead was being excreted without treatment, potassium iodide caused its appearance in the excreta, and in those cases in which lead was present during the control periods, the total excretion was doubled—an average

excretion of 0 68 mgm (ten periods) rose to 1 29 mgm (28 periods) This increase continued during the first period after potassium iodide was discontinued

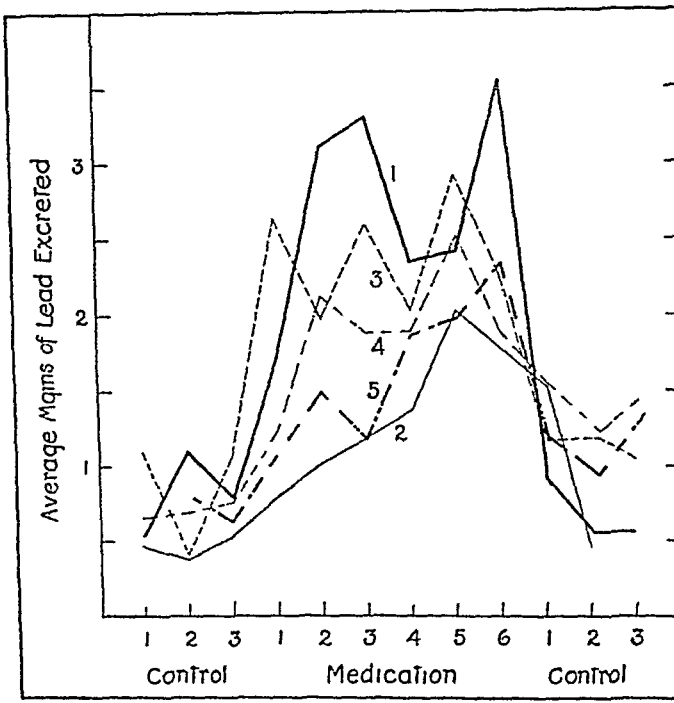


FIG 20 SUMMARY OF EFFECTS OF MEDICATION

Each curve represents the average excretion of lead for consecutive three day periods

Curve 1 The effect of phosphoric acid and a low calcium diet The first control represents only two observations

Curve 2 The effect of phosphoric acid and a normal diet. The first and last control periods represent only two observations each

Curve 3 The effect of ammonium chloride

Curve 4 The effect of sodium bicarbonate The last medication period represents only two observations

Curve 5 The effect of potassium iodide The fourth, fifth, and sixth medication periods, the high points in the curve, represent only single observations

A few experiments were performed in an attempt to determine whether it was the potassium or the iodide ion which produced this effect Potassium chloride was administered in three experiments (5 grams daily) without obvious change in rate of excretion. The action of ammonium iodide was then tested because in the body ammonia would largely form urea which should exert no effect (see also ammonium phosphate experiments). Our one observation indicated that the definite increase of lead excretion was hardly less marked than with the potassium iodide which followed (see table 31) These experiments therefore suggest that it is the iodide ion which influences lead excretion

Although potassium iodide has long been used empirically with fair success in the attempt to increase excretion of lead, its mode of action is unknown The determination of various factors involved in the mobilization of lead, and therefore of effective methods for its control, has seemed desirable. Therefore, the medications tested on animals were further studied in man

Acids. The effect of acids must be dependent upon (*a*) their toxicity and (*b*) their destruction in the body. The toleration of the organism to them apparently varies with the strength of the acid. Far larger quantities of phosphoric than of hydrochloric acid may be taken without discomfort Ammonium salts, however, which Haldane (183) has shown to be effective in producing acidosis, may be administered with far more ease than any of the acids Although *in vitro* certain acids dissolve lead more readily than others in equivalent concentration (hydrochloric, phosphoric, lactic, and tartaric acids, in order of increasing solvent action), it is not to be expected that this would be true in the body, for in the organism acids must be neutralised or be burned The organic acids which are burned must have less effect on the hydrogen ion concentration of the organism and on the solubility of lead unless they are locally produced Lactic or citric acid, for instance, when taken by mouth should theoretically be ineffective

In treating patients in the hospital, results were obtained which seemed to bear out these differences Daily administration of about 140 cc of N/10 hydrochloric acid in four cases raised the average excretion from the control level of 0 31 to 0 88 mgm per period, and

the rate of excretion fell to an average of 0 28 mgm after the acid was stopped (see fig 20 and table 31) These were early experiments, however, and were not as satisfactory as later observations

Phosphoric acid was administered with diets of both high and low calcium content Twelve observations were made with high and ten with low calcium intake During most of the control periods the diet contained much calcium In nearly all of the individual experiments the excretion of lead increased during the ingestion of acid Figure 20 demonstrates the average curves obtained from all the observations [1] The striking increase in excretion of lead in both series is shown in

TABLE 32

The daily urinary excretion of phosphorus

	PATIENT		
	HS	PC	BL
	gm	*gm*	*gm*
Before acid	1 47	1 20	0 47
	1 53	1 25	0 49
	1 58	1 00	0 36
			0 73
During ingestion of phosphoric acid	3 47	2 52	2 08
	3 16	2 46	2 04
	2 26	2 84	2 05
		2 36	1 88

table 31 Apparently acid with a low calcium diet caused a greater excretion of lead than occurred when the diet was normal This is more striking than the chart shows, for the average control figure is elevated because eight of the nineteen control observations were made during a low calcium intake In these, the average excretion per period was 1 27 mgm as compared with that of 0 48 mgm during the eleven control periods when the diet was rich in calcium

That the phosphoric acid was absorbed by the gastro-intestinal tract was determined in several ways The quantity of inorganic phosphate in the urine when the diet was constant proved much

[1] These curves do not represent the same number of experiments in each period, because all observations were not equally prolonged Accurate comparisons can best be made by reference to the data of each observation which is given in our original paper

greater than normal (see table 32) and the increase could only have been derived from the acid absorbed Determinations showed that the quantity of urinary ammonia gradually rose to a high level during acid ingestion The CO_2 combining power of the blood plasma was definitely reduced during ingestion of acid in a majority of our numerous determinations Dr A O Koehler has also demonstrated that the hydrogen ion concentration of the blood fell during acid ingestion to a figure as low as 7 19. As soon as administration of acid ceased, these figures returned promptly to their previous normal values

In an effort to produce acidosis without the discomfort of direct ingestion of acid, the ammonium salts of both phosphoric and hydrochloric acids were administered (183) Ammonium phosphate ($(NH_4)_2HPO_4$) proved rather ineffective in two cases It increased the average excretion from 1 27 mgm per three-day period before medication to 1 60 mgm , but the average excretion fell to 0 66 mgm of lead per period after medication ceased Ammonium chloride, on the other hand, when administered with a diet of low calcium content, gave much more satisfactory results (eight cases), in fact it appeared to be as efficient an agent as phosphoric acid The daily dosage varied somewhat, but could be as large as 12 grams Just as during treatment with phosphoric acid, the hydrogen ion concentration of the blood plasma fell and the CO_2 combining power was diminished while this salt was being ingested Increased quantities of ammonia gradually appeared in the urine under this regime Thus it is demonstrated that ammonium chloride produces acidosis quite comparable to that caused by phosphoric acid

Alkalies. Because of the greater solubility of tri-lead phosphate in alkaline as well as acid medium, attempts were also made to increase elimination of lead by rendering the tissues more alkaline than normal Sodium bicarbonate (in doses up to 40 grams per day) given with the usual varied diet produced striking results in seven cases (table 31 and fig 20) It reduced the urinary excretion of nitrogen, increased the CO_2 combining power of the blood plasma, and changed the hydrogen ion concentration to pH 7 55 Although sodium bicarbonate does not cause as great an increase in the rate of elimination of lead as do the acids or acid-forming substances, it possesses certain advantages, for it was given without a special diet and seems to produce

an effect which continues longer after cessation of treatment than that caused by other forms of medication In one observation sodium citrate was found to increase only slightly the rate of excretion (see table 31)

As all of these drugs were ingested in quantities which approached the limit of tolerance, it was necessary to watch the general condition of patients with care Usually they lost appetite, and during the development of acidosis sometimes suffered from headache and general malaise The weight which was lost during treatment with acids was rapidly regained during control or rest periods Ammonium chloride and sodium bicarbonate seemed to have less general effect Occasionally medication produced diarrhea In no case, however, was it the cause of an acute manifestation of poisoning, and in patients suffering from wrist drop satisfactory improvement progressed Under the regimes thus outlined, recovery from anemia was rapid, and the stippling of red blood cells gradually disappeared The general condition of the patients was excellent and they regained strength, gained weight, and recovered good color

In order to give a clearer picture of our results, the charts of three patients are included These show the effect of diet and medication upon the excretion of lead for a long time and include only the more important data Such various factors as calories or type of food, salt intake, and volume of excretion, which apparently exert but little if any influence on the lead excretion, are omitted The longer observations were made on individuals who, because they were temporarily disabled by bilateral wrist drop, could be kept in the hospital indefinitely

In looking over the charts it is important to observe not only the effect of the medication but also that exerted by the calcium intake and balance

M G H 259852 John L , thirty-two, Austrian, chauffeur

Nine days before entrance he had a chill, fever, and pain in joints, with persistent constipation Two days ago he developed severe generalized abdominal cramps This is his second such attack

Physical examination showed a pale man with a marked lead line The abdomen is soft but tender in epigastrium, and the liver edge is palpable and tender There is no muscle weakness.

Laboratory findings· Red blood cells 3,600,000 Hemoglobin 65 per cent White count 8000 The reticulated red cells were 3 to 6 per cent, and many stippled cells were found The serum dilution of his blood was elevated to a level of 1·40, and bile obtained by duodenal tube showed increased amount of bilirubin, both of which suggested increased blood destruction X-rays of lungs, gall bladder and gastro-intestinal tract were not remarkable Blood pressure 140/70

Course· He continued to complain of some right upper quadrant pain until it was definitely decided that his gall bladder was normal The source of the lead was not discovered He left the hospital feeling well, having gained six pounds

The treatment and lead excretion is shown in fig 21

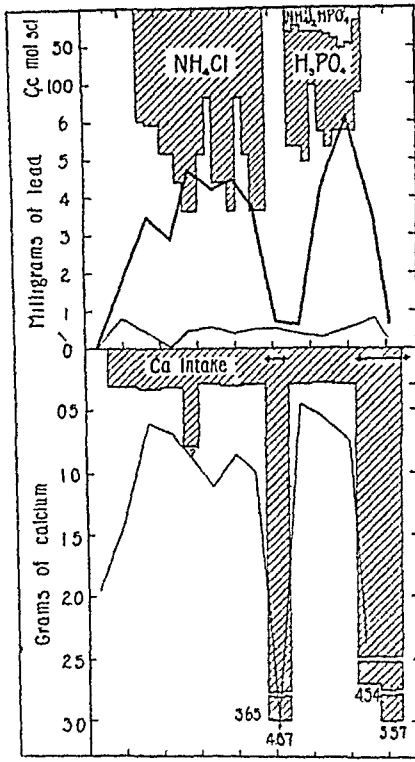
M G H 260749. John T, thirty-four, Lithuanian, rubber mixer for five years

Three months ago he developed severe colic with marked constipation and vomiting Then he noticed weakness of wrists, which was most marked in the morning He worked until three days ago, when he was too weak to continue Suddenly he became maniacal and had a convulsion and was taken to the Psychopathic Hospital He was pale, wildly maniacal, showed marked confusion, was completely disoriented and violent, and had hallucinations of sight and smell

Physical examination showed marked pallor and lead line He had a bilateral wrist drop with involvement of upper arms of the brachial type of Remak, involving deltoid and supraspinatus muscles on the right and left, though palsy of deltoid and supraspinatus muscles on the left was not complete. There is definite swelling in the tendon sheaths of the paralyzed wrist muscles

Laboratory findings Red blood count 3,800,000 with marked stippling of blood cells Spinal fluid was normal, except for increased pressure, though lead was found in it The Wassermann test was negative in blood and spinal fluid The renal function, non-protein nitrogen, blood calcium, phosphorus and sodium chloride were all normal

Course: Patient recovered completely from mania within twenty-four hours after high calcium intake was started In three weeks he was transferred to the Massachusetts General Hospital, and was kept for a long time because he was economically completely disabled Gradually strength returned in his hands and arms, and at discharge (after seven months) there was good movement in all muscles though the extensors of the wrist



FIGS 21, 22 AND 23 THE EFFECTS OF MEDICATION AND DIET UPON LEAD EXCRETION IN INDIVIDUAL CASES

The interval marks on the abscissa represent five days

Above the middle horizontal line is shown the medication and lead excretion The faint line is the excretion of lead in the urine expressed in output for three days The heavy line is the total excretion of lead in the urine and feces for three day periods The medicaton is shown in blocks representing the cubic centimeter of molar solution taken per day These really represent as much as the patient could take without toxic symptoms

Below the middle horizontal line is shown the calcium intake in blocks of three days total ingestion The arrows in areas of high calcium intake represent the addition of milk or calcium lactate to the routine low calcium diet The areas which have no enclosing block line represent periods of unweighed full diets The single line represents the total calcium output in urine and feces in three day periods The distance between line and blocks therefore represents the positive or negative calcium balance

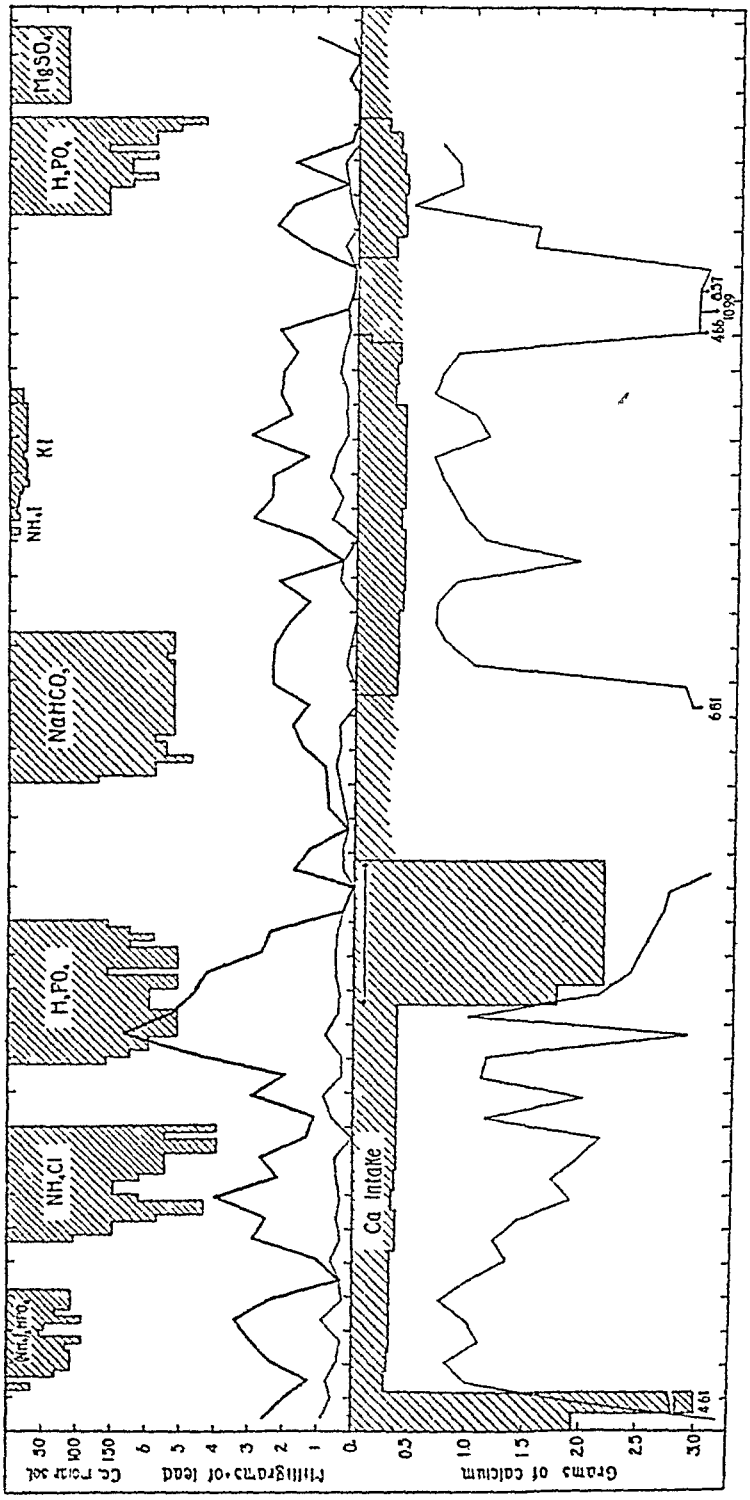

MgSO4
H3PO4
KI
NH4I
NaHCO3
H3PO4
NH4Cl
Ca Intake
Cc. Polar sol.
Milligrams of lead
Grams of calcium
50
100
150
6
5
4
3
2
1
0
0.5
1.0
1.5
2.0
2.5
3.0
461
661
466
1099
837

FIG 22

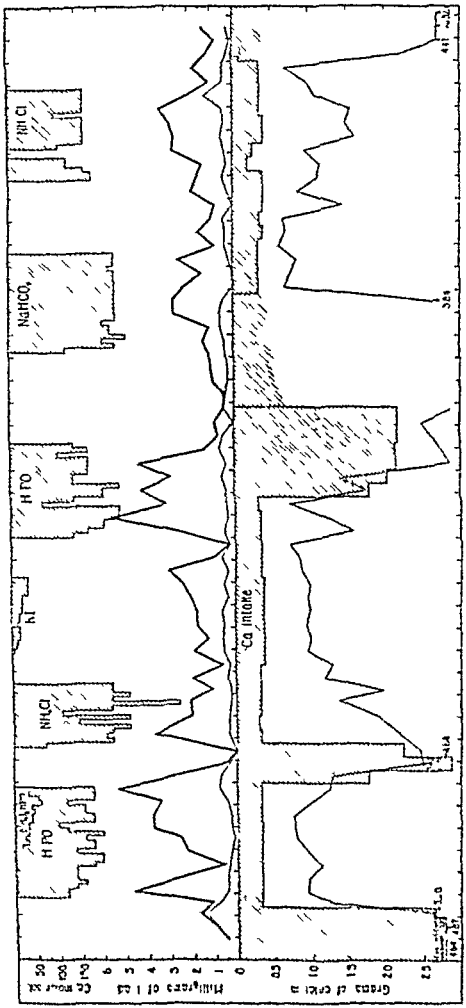


Fig 23

were still not strong. During his 204 days in the general hospital he felt well, complained of no colic, and gained twenty pounds in weight.

His treatment and lead excretion are shown in figure 22

M. G. H 259296 Pando M , forty-eight, Greek, rubber mixer for seven years

For five months he has had repeated attacks of severe abdominal colic. For four months he has felt "burning" sensations of feet and easy fatigue. Seven weeks ago his hands and arms felt "burning," and his hands became weak The ring fingers were first involved, then the middle, and later the index finger, and finally the wrist The palsy in the right hand was more severe than the left.

Physical examination showed a pale, wan-looking man with a marked lead line His arms showed no sensory disturbances, but great weakness in all movements, particularly in biceps and deltoid, and there was a typical double wrist drop which was more marked on the right Both legs were weak and there was a slight but definite toe drop on the right There is definite swelling in the tendon sheaths of the wrist muscles which are paralyzed

Laboratory findings Hemoglobin 75 per cent Red blood cell count 3,700,000, with marked stippling of the cells Non-protein nitrogen of blood was 51 mgm per cubic centimeter, but the calcium, phosphorus, and chlorides were normal and the sulphone phenolphthalein excretion averaged 50 per cent in two hours.

Course Colic stopped promptly on entrance, and in two months his upper arms had markedly regained strength The wrist drop gradually improved, and at discharge, both hands were still weak, but all movements could be made He felt well except for continued burning sensations in his legs The red cell count was 4,900,000 and there was no stippling found He gained twenty-two pounds during his 237 days in the hospital

His treatment and lead excretion are shown in figure 23

Summary. The various observations on both cats and man seem to demonstrate that both acids, acid-forming salts, and alkalies definitely increase the excretion of lead to a greater extent than does potassium iodide Magnesium sulphate has apparently no such effect Although acids increase excretion of lead when the diet is normal, they are much more effective when the calcium intake is low. Sodium bicarbonate and potassium iodide produce their action without a

special diet In potassium iodide it is apparently the iodide ion which is effective

PART II PATHOLOGY

X PATHOLOGY

The lack of correlation between the clinical picture of lead poisoning and the pathological findings has produced much controversy Patients who have presented many symptoms suggestive of pathological change usually show at post mortem examinations little of interest Moreover, the many ante mortem symptoms and signs are explained by the autopsy with great difficulty and lead is rarely proved to be the immediate cause of death (3) Under such conditions the confusion in the literature may readily be imagined Not only have many opinions been based on the examination of isolated cases, but incorrect conclusions have been drawn from autopsies which revealed lesions characteristic of conditions other than lead poisoning Facts such as these probably led conservative and careful clinicians to sound a warning that appendicitis as well as colic can attack a lead worker and that those who have absorbed lead are not immune to cerebro-spinal syphilis

Even experimental lead poisoning has done little to clarify the situation Many of the investigations have been uncontrolled and certain of the pathological conditions attributed to the action of lead may frequently be seen in supposedly normal animals No doubt some work suffered from attempts to demonstrate a relation between the lesions found in an organ and the quantity of lead recovered from it This, of course, led to the accumulation of much contradictory evidence and complicated the question by a fruitless discussion as to whether lead acts directly or indirectly Some investigators believed that lead so affected the organism that an abnormal metabolism resulted and permitted the formation of toxic substances

Since our patients have shown little that can be definitely ascribed to the action of lead, and since microscopic examinations of the tissues from our animals have not yet been made, the main purpose of the following account is to review the literature in so far as it in-

cludes observations which have been made with some regularity, and have been confirmed, or which have an obviously important bearing on the subject. In addition, in order to avoid increasing the existing confusion, an attempt will be made to evaluate these observations so that some idea may be obtained of the relative importance of the lesions attributed to lead

Students have always endeavored to crystallize into a pathological entity the isolated observations of each clinical condition To this end wherever possible they have sought to attribute a specific lesion to each etiological factor Lead has been no exception

The specificity of the lesion in lead poisoning has been most strenuously advocated by Legge, Goadby, and Goodbody (252) (166) These workers claim that the specific lesion in lead poisoning consists of hemorrhages caused by the action of lead on the vessels, especially the minute venules Following this condition secondary pathological changes may occur, particularly fibrosis, as was suggested previously by Siccardi (430) (431) and also Hitzig (218) This idea of a characteristic change in plumbism was based on results in some animal experiments and more particularly on a case reported by Mott (321). There are many objections to this view One of the most frequent signs of lead poisoning is anemia, often of marked degree (section XVII). This alone is sufficient to explain the occurrence of hemorrhages Moreover, Mott's patient had nephritis and arteriosclerosis, conditions frequently associated with hemorrhages Mott definitely states that many of the lesions were probably artefacts. Catalano (72) also believed that hemorrhages constantly accompanied lead poisoning, but his patient suffered from convulsions, and there is no reason to believe that this symptom may not have been a contributing cause rather than the result of hemorrhage Siccardi performed experiments on isolated arteries, perfusing them with salts of lead He found that these vessels shrank markedly and concluded that lead acts on the smooth muscle of the media These tests, however, were performed entirely *in vitro* and with very concentrated solutions of lead Their applicability, therefore, to conditions *in vivo* is not established In sections of liver, which we perfused with lead salts, the lead was entirely taken up by the endothelial cells of the vessels, none penetrating into the media. The evidence offered for the theory of vascular injury, however, has not changed the opinion of most observers, which holds that there is no lesion characteristic of lead poisoning, except perhaps the lead line.

General appearance At post mortem examination, patients who have had lead poisoning present the appearance of marked undernourishment which is emphasized by absence of subcutaneous fat

Mucosa Probably the most characteristic and common finding is the so called lead or Burtonian line—a dark line on the gingiva near the border of decaying teeth This is not a superficial formation, for it cannot be rubbed off Microscopic examination of small sections snipped from the gums shows that the lead line is composed of fine black amorphous granules which lie in the connective tissue at the base of the epithelium These are also found in the interior and in the walls of vessels, in connective tissue fixed cells, and in macrophages A similar black discoloration in the mucosa of the colon and in the lower portion of the small intestine, first observed by Tanquerel (453), is sometimes seen

Gastro-intestinal tract The common occurrence of colic in plumbism has focused the attention of pathologists on the gastro-intestinal tract and several lesions have been reported

In acute fatal cases, Hutton (222) states that entero-colitis results from the caustic action of lead, while in chronic cases a long continued gastro-enteritis, consisting of glandular atrophy of the stomach and small intestine, and intertubular diffuse sclerosis, has been frequently observed (299) (243) Legge and Goadby have noted a slight degeneration of the muscular coats with infiltration and minute hemorrhages Maier (274), who also reported finding hemorrhages in the gastro-intestinal tract, contributed to this list of changes a fatty degeneration of secretory cells, enlargement of arteries, venous stasis, and circumscribed areas of softening Of special clinical interest are the experiments of Jores (230) in which he produced gastric and intestinal ulcers in dogs by the administration of lead These are significant because of the frequent occurrence of ulcers in lead intoxication, and may suggest a method of investigating the mechanism by which such lesions are formed

Various pathological changes have been reported as the underlying cause of colic Maier believed that sclerotic degeneration of the submucous and myenteric plexuses is the fundamental factor in its etiology This abnormal condition of the sympathetic innervation of the gastro-intestinal tract has also been reported by Tanquerel who described hypertrophy of the coeliac ganglion, and by Mosse (320) who thought that sclerosis of the sympathetic ganglion was always the cause of colic Both Tanquerel and

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Oliver (337) have reported that the intestines are contracted when colic has occurred before death, but no description of the lesion actually responsible for this condition has ever been given Alcock, moreover, says that he has never observed the contraction in human autopsies, although he frequently found it in his guinea pigs Meillère (293) believed that lead may cause a general hypertrophy of all glandular appendages of the gastrointestinal tract followed by atrophy which gives the senile appearance to the tissues; but he emphasized that most of the lesions described by investigators might very well be due to alcohol and that their significance in the diagnosis of lead poisoning should be questioned.

Liver. The rôle played by lead in the production of hepatic lesions is of considerable interest because of the importance of the liver as an eliminator of lead, especially of lead which enters the system by way of the mouth, and because of the supposed relationship of lead to the production of cirrhosis of the liver

Several types of lesions of precirrhotic or cirrhotic character have been described Hutton, Potain (364), and Alcock have reported the occurrence of atrophy of the liver cells, fatty degeneration or infiltration, and intercellular or interlobar cirrhosis, and Legge and Goadby add that small areas of exudation and hemorrhage may also be seen Kussmaul and Maier believed that lead acts directly on the portal vessels, but Hanot and Charpentier (197) thought that the hepatic cells themselves were directly affected Meillère states that periportal cirrhosis may be produced experimentally by lead, but Ophuls (340), who worked with guinea pigs, never saw this lesion In his animals a marked necrosis of cells was followed by a collapse of the tissue and dilatation of veins, which he called "chronic focal atrophy." To this he never attached much importance, for he believed that the only condition attributable to the action of lead was hematogenous pigmentation of the organ. With very large doses of lead acetate Mallory (277) produced basophilic instead of acidophilic hyaline lesions within the hepatic cells, necrosis and phagocytosis of these cells, and saw evidences of regeneration in the mitotic figures within some of them Striking definite cases like that reported by Seeligmuller (412) are rare He found evidences of hepatic cirrhosis in the liver of the new-born child of a lead worker and recovered lead from the organ

The relationship of lead to so-called alcoholic cirrhosis has been the subject of much controversy. It is a far cry from Chauffard's

(78) belief that all cirrhosis of the liver in plumbism is really caused by over-use of alcohol, to Mallory's statement that all cirrhosis attributed to alcohol may be due to contaminating lead In spite of all the pathological conditions which have been noted in plumbism, the consensus of opinion among pathologists who have studied lead poisoning confirms Oliver's view that post mortem examination of the liver reveals no changes specific for lead

Heart The heart, as might be expected from clinical observation, shows nothing remarkable as a result of lead intoxication Such cardiac lesions as hypertrophy, secondary to supposed vascular and renal changes (347), myocardial degeneration following hemorrhage (252), and even brown atrophy (243) have been described, but most observers believe that the heart undergoes no real change, certainly none due to the direct action of lead (337)

Vascular system With respect to the effect of lead on the rest of the vascular system, however, there is much less agreement

As previously mentioned, the blood vessels have been considered the principal point of attack of lead chiefly because of the work of Legge and Goadby, who described the occurrence of perivenule hemorrhages But even before these investigators presented their view, Henle (208), Heubel (213), Maier (274), Hitzig (218), Rosenstein (390), Elschnig (114), and others believed that the vascular system was first involved Elschnig thought he could demonstrate an actual degeneration of the unstriped muscle of the vessels Even arteritis, sometimes to an obliterating degree, has been reported (463) In this connection it is of interest to note the case cited by Kazda (232) of a patient with gangrene of the legs supposed to be due to lead arteritis Despite all this evidence and the fact that Gouget (175) observed aortitis in one of his guinea pigs, it is noteworthy that Ophüls never was able to produce vascular lesions experimentally Although the general clinical opinion holds that arteriosclerosis is a result of chronic plumbism, this idea is based entirely on the occurrence of this condition in many post mortem examinations That arteriosclerosis is much more frequent in those exposed to lead than in other individuals of the same age, however, needs much more statistical proof before it can be accepted

Kidney The effect of lead on the kidney is of very great practical importance both with respect to nephritic conditions and also because of the possible uremic origin of encephalopathy Two types

of lesions have been reported, an acute and a chronic nephritis. Oliver describes an acute tubular nephritis characterized by cloudy swelling and fatty degeneration of the tubules with destruction of the cells. Occasionally cellular proliferation in glomeruli appeared with leucocytes gathered around the afferent vessels. In some cases (222) an atrophy of the glomeruli with hyaline degeneration of the vessels has been seen The chronic nephritis supposed to be caused by lead presents the picture of the typical secondarily contracted kidney.

Charcot and Gombault (76) think the lesion is primarily an epithelial cirrhosis. On the other hand, Legge and Goadby maintain that such a condition in lead workers has a vascular origin, but suggest that kidney changes have never been directly traced to lead and are probably due to alcohol. Such a stand is of interest in view of the fact that Oliver, who is so conservative in his conclusions, states quite definitely that in chronic plumbism chronic interstitial nephritis with small contracted kidneys and thickened arterioles is a frequent finding.

Experimental work has not yet succeeded in deciding just what part lead plays in establishing nephritic conditions. Experiments were carried out by Ophuls who used guinea pigs in an effort to produce renal lesions He found pathological changes in the distal convoluted tubules, consisting of granular degeneration, pyknotic nuclei and necrotic cells There were also many epithelial casts and some evidence of regeneration of the epithelium. However, he very significantly points out that this entire picture was frequently seen in his control animals He therefore holds that the only constant, but very limited, lesion is some degeneration and fibrous thickening of the glomeruli which he did not see in the normal guinea pigs

The only attempt to study "lead nephritis" statistically has been made by Machwitz and Rosenberg (272) They investigated the relative incidence of Bright's disease in lead workers compared to other laborers and found that of thirty-six cases of "malignant sclerosis" seventeen occurred in laborers, nine of whom had worked with lead They also quote Volhard who reported that four of his thirty-six patients with Bright's disease were lead workers These are the only available statistical reports of the incidence of nephritis in lead workers and because the figures are so scanty no definite conclusion can be drawn from them Here, as in the case of arteriosclerosis, the accepted clinical opinion that lead can cause chronic interstitial nephritis is far from convincing Certainly the urinary, blood pres-

sure, and blood chemistry findings hardly point to a marked kidney involvement This is all the more interesting because of urinary excretion of lead

Brain The severity of lead encephalopathy with its uncontrollable symptoms and high mortality makes the question of the action of lead on the nervous system of more than passing interest A full discussion of the possible pathogenesis of encephalopathy will be given in section XVI, but in this section only the different pathological pictures described by various observers will be reviewed

The brain has been seen by various workers to be shrunken, firm, pale, sometimes edematous, often icteric, and the convolutions may be flattened or atrophied (453) (293) (337) Considerable attention has been paid to the microscopic picture of the brain in encephalopathy Hutton mentions that arteriosclerosis and arteritis may be found, and Mott, Legge and Goadby, and Courtney (85) have observed disseminated hemorrhages in the perivascular spaces after chronic encephalitis They thought these were caused by arterial and venous degeneration, and considered them the characteristic lesion of this condition Mott, in his case, saw marked hyperplasia of the neuroglial cells Round cell infiltration has also been noted, but frequently when the picture is complicated by syphilis, as in two of our cases

Perhaps the most important contribution to this subject has been the examination of the cerebro-spinal fluid of encephalopathic patients by Mosny and Malloizel (316), Norton (332), and Boveri (50)

These investigators report a constant pleocytosis (approximately 100 lymphocytes per cubic millimeter cerebro-spinal fluid), an increased quantity of globulin, and increased pressure Hassin's (203) careful pathological examination of brains after uncomplicated lead encephalopathy gave results which fit in well with these observations He saw marked proliferation of fibrous tissue and blood vessels in the pia-arachnoid space, especially near the cerebellum, optic chiasma, and temporal lobe, and infiltration by lymphocytes and plasma cells in the relatively acute cases The parenchyma was only very slightly affected, and was described by Hassin as follows "The glia cells, like the pia-arachnoid, showed marked progressive changes, increased size of nuclei, abundance of chromatin and cytoplasmic processes, and a great proliferation of glia nuclei gathered in rows or clusters

They usually contained fat and frequently invaded the ganglion cells Most of the latter were well preserved, some showing chromatolytic changes, vacuolation and neuronphagic phenomena " He found that although this proliferation of blood vessels was most marked in the meninges and choroid plexus, it occurred to some extent in the cord, cerebellum, mid-brain, cortex, subcortical white substance, and large ganglia, and was not only an endothelial and adventitial overgrowth, but also a formation of new capillaries Signs of perivascular infiltration were, however, strikingly absent After a consideration of all these microscopic findings Hassin concluded that lead encephalopathy contrasts sharply with infiltrative, infectious encephalitis In plumbism the meninges, the base of the brain, the cerebellum, the optic chiasma, and the temporal lobe are involved, while epidemic encephalitis affects chiefly the mid-brain In his opinion round cell infiltration in lead encephalitis is merely the manifestation of a reaction against the irritating substance, and is characteristic of the more acute form of the disturbance It is interesting that Hassin makes no mention of the hemorrhages which play so large a part in many neuropathological descriptions of lead encephalopathy

The question, therefore, arises whether lead encephalitis is not really a meningo-encephalopathy, which is primarily a chronic productive meningitis, characterized during acute exacerbations by a degree of round cell infiltration varying with the severity of the attack. Certainly most of the recent pathological and clinical pathological observations seem to bear out this theory.

Spinal cord The occurrence of a type of amyotrophic lateral sclerosis in lead poisoning makes a study of the pathology of the spinal cord of value Gross changes have never been observed (453), but many microscopic alterations have been found A change most constantly seen and most closely related to the clinical picture of muscular atrophy is the degeneration of the anterior horn cells (210) (443) (293) (321) This is characterized by vacuolation of the ganglion cells, perinuclear chromatolysis, fatty pigmentation, and marked shrinking of the cells

Hutton reports that following degeneration of nerve fibers there is an increased neuroglosis and proliferation of connective tissue Catalano, and Legge and Goadby describe the occurrence of punctate hemorrhages within the cord also, and Hitzig states that inflammation of blood vessels

is an important and common lesion A lymphocytic infiltration around the adventitia of the arteries has been reported by Mott, and some observers have found that distention and actual alterations of the venules may be seen The older investigators believed that nuclear proliferation occurred frequently (313) (406) (490)

In cases of atrophic paralysis, therefore, the lesion is probably amyotrophic in character and may perhaps include some sclerosis of the lateral columns

Peripheral nerves Perhaps no question in the pathology of lead poisoning has been the subject of so much controversy as the site of the action of lead in palsy Although typical lead paralysis is not of the atrophic type just described, but resembles so-called peripheral neuritis, it has been attributed by some authors to cord lesions However, the consensus of opinion seems to confirm the peripheral location of the lesion and is based upon the observation of very distinct changes in the peripheral nerves themselves

The older writers, especially von Monokow, Schultze, Westphal, and Eichhorst (108), were impressed with nuclear proliferation especially around the vessels Eichhorst also believed that the blood vessels were thickened and thought, as did Meillere and Dejérine-Klumpke (92), that the essential lesion was a homogenous degeneration of the sheath of Schwann—a typical Wallerian degeneration Dejérine-Klumpke, quoting Gombault (171), emphasizes that the lesion is essentially peri axial in character, and that the axis cylinders themselves are not involved Except for Legge and Goadby, who found minute hemorrhages in the peripheral nerves, most workers agree that the usual picture is that of atrophic, degenerative neuritis with subsequent fibrosis

The broad significance of these pathological changes will be discussed with the probable mechanism which produces lead palsy

Muscles Because of its importance in the explanation of lead palsy the pathological condition of the muscles merits careful consideration Varying degrees of atrophy have been observed by most workers (293) (247) (111) (199) (149) (231), and this condition is generally accepted as a constant accompaniment of lead paralysis (224) It is, however, of considerable significance that these muscles do not merely undergo simple atrophy, but that the changes are

quite similar to those seen in the nerves which supply the affected muscles.

Thus, Legge and Goadby found minute hemorrhages scattered throughout the muscle, Goadby (252) saw fatty degeneration, and nuclear proliferation of the perimysium and loss of cross striations and granular degeneration have been described by various authors (111) (171) (156) (83) (406). Most investigators who have examined the muscles agree with Messing (301) that the lesion is a chronic, degenerative, parenchymatous myositis, with fibrous tissue replacement, and that the changes are not merely secondary atrophy following nerve lesions, but are caused directly by the action of lead

Blood A detailed description of changes in the blood during lead poisoning more properly belongs in the chapters dealing with the clinical and physiological aspects of the disease, and will, therefore, be omitted here But at this point the observation first made by Laennec (244) of the pallor of body tissues after death from lead poisoning and of a diminution of blood in the vessels should be mentioned

Spleen As might be expected from the marked hemolytic action of lead, the spleen frequently shows increased pigmentation due to destruction of blood (177) (230). Legge and Goadby state that in their animals the venous sinuses of the spleen were distended with blood. Galvini (151), the only other investigator who reports changes, describes the occurrence of chronic atrophy Oliver believes that there are no noteworthy changes in this organ due to plumbism.

Bone marrow Until recently the entire subject of lead anemia has been quite unexplained Lesions of the bone marrow have been suspected as the underlying cause of this condition

Stockman and Charteris (445), by administering lead to animals, produced a decrease in the number of fat cells and, after very large doses, observed gelatinous degeneration of the bone marrow. Except for Raimondi (373), no other investigators have obtained such degeneration In fact, Cadwalader (60) in his one patient, and Ophuls in all his animals, report that a hyperplasia occurred which included the myelocytes, nucleated red cells, and occasionally the megaloblasts Ophuls also states that there

is increased hematogenous pigmentation of the bone marrow during lead poisoning Wolff (496) has given a similar interpretation of the changes He believes that the number of young blood cells increases within the marrow because of a sudden and excessive demand occasioned by peripheral destruction of the blood cells, and he gives no evidence that lead injures the marrow directly In the light of the clear experimental evidence for destruction of circulating red blood cells during lead poisoning, this hypothesis is of importance

Bone The storage of lead in the bones appears to have little or no effect on their structure Various pathological conditions have been described from time to time but none of them has been proved to have any direct relation to the action of lead on skeletal tissue

Tanquerel, by gross inspection, could find no abnormal conditions in the joints to explain the frequent development of arthralgia during plumbism Lewy (264) believed that lead could cause necrosis of the bone, and his belief is confirmed to some extent by the common occurrence of caries of the teeth in lead workers Osler reported that gouty deposits in the big toe joint might be seen in cases of lead poisoning and thought that the incidence of gout was higher than normal among people who had been exposed to lead

None of these observations demonstrates that there is any consistent alteration of bone during plumbism, and this seems to support the physiological view that while lead is stored in the bones it is harmless

Lungs The supposed prevalence of respiratory diseases among lead workers, particularly of pulmonary tuberculosis, might lead one to expect frequent lesions of the lung in individuals exposed to lead

Lewy believed that acute asthma, characterized by degeneration, inflammation, and necrosis of the bronchial mucosa, was a common form of trouble in lead poisoning and stated that this disturbance frequently develops into chronic bronchitis, and Greven described endarteritis in the lungs of his rabbits Gibert (164) has also reported that lesions may appear in the lungs as the result of exposure to lead

But by far the greater proportion of evidence tends to show, in agreement with Oliver's statements, that no typical pathological changes occur in the lungs as a result of the action of lead

Generative organs Like syphilis, lead may cause sterility, abortion, and defective issue Although exposure of either parent to lead may produce these results, no pathological changes have ever been noted in the ovary In the male, on the other hand, gradual disappearance of spermatozoa from the gonad, and degeneration and atrophy of the germinal epithelium are frequently seen (486) (32). This picture may occur, however, in many conditions other than lead poisoning Bell reports that lead causes degeneration of the chorionic epithelium This, in addition to the action of lead on the uterus itself, must be considered a cause of abortion which is so common among lead workers and among women who take diachylon to cause this condition

Salivary glands A few isolated observations have been made on the condition of the salivary glands in lead poisoning

Apert (11) believes that lead causes a simple hypertrophy of the parotid gland and occasionally inflammatory lesions of the ducts Rarely periglandular sclerosis has been seen Allevi (7) thinks that infection of the gland by way of the mouth is a frequent complication of lead poisoning Lesions in the other salivary glands have never been described

Glands of internal secretion Very little has been published about the effect of lead on the glands of internal secretion, probably because these had not attained their present importance at the time when most of the pathological studies were made The only lesion described in the literature is hypertrophy of the suprarenal cortex (35) (74) (175)

Special senses The eye is the only sense organ in which lesions have been found in lead poisoning Such various pathological changes as retinal hemorrhages and acute neuro-retinitis, characterized by swollen, hyperemic discs have frequently been observed Optic atrophy, however, is the most commonly described lesion (222) (454)

Gibson (157) believes that this condition is due to increased cerebrospinal pressure Mosny and Harvier (317) report the occurrence of retinal exudation in one case, but they thought that this might be merely the manifestation of a complicating albuminuric retinitis Some of the temporary amblyopias have been ascribed to spasms of the blood vessels

Serous membranes No reports of pathological conditions in the serous membranes of man during lead poisoning have been published in the literature, but Ophuls has produced experimentally a sero-fibrinous polyserositis This he believes is caused by increased permeability of the blood vessels and fibrous proliferation

Site of injection of lead Kumita (242) states that at the site of injection of lead salts calcification occurs in rabbits

Conclusion No classification of the various lesions caused by lead can escape criticism, but it seems wise to group the various findings with conservative caution into the four following classes (*a*) lesions which are found constantly and are caused by lead, (*b*) lesions which frequently occur and are caused by lead, (*c*) lesions frequently seen in subjects with evidences of lead absorption, and (*d*) lesions which have been reported to occur occasionally in subjects with evidences of lead absorption and which may possibly be caused by lead Under these headings the pathology of lead poisoning may be summarized as follows

A Lesions found constantly, due to lead
  1 Blue line
  2 Anemia and stippling
B Lesions found frequently, due to lead
  1 Degeneration of anterior horncells
  2 Peripheral neuritis
  3 Chronic muscular atrophy and fibrous myositis
  4 Degeneration of male gonads
  5 Decrease in fat cells of bone marrow and hyperplasia of leucoblasts and erythroblasts
  6 Productive meningitis
  7 Blue patches on mucosa of large and lower part of small intestine
  8 Optic atrophy
C Lesions seen frequently in people with evidences of lead absorption
  1 Arteriosclerosis
  2 Ulcers of stomach and intestine
  3 Contracted small intestines
  4 Tubular nephritis or chronic interstitial nephritis
  5 Hemorrhages and exudate of retina and neuro-retinitis
D Lesions which have been reported to occur occasionally in people with evidences of lead absorption or lesions possibly due to lead

1. Vascular hemorrhages
2 Cardiac hypertrophy, hemorrhages, brown atrophy
3. Degeneration of unstriped muscle of vessels
4 Perivenule hemorrhages
5. Arteritis
6 Gangrene of extremities
7 Entero-colitis, acute or chronic
8 Hypertrophy or sclerosis of sympathetic ganglia
9. Inflammation and infection of parotid gland
10 Degeneration, atrophy, cirrhosis of liver
11 Hematogenous pigmentation of liver and spleen
12 Hypertrophy of spleen due to erythrophagia or atrophy
13 Hemorrhagic encephalitis
14 Neuroglial hyperplasia
15 Cerebral and spinal cord lymphocytic infiltration
16 Hemorrhagic myositis, fatty degeneration
17 Increased pigmentation of bone marrow and gelatinous degeneration
18 Acute or chronic bronchitis
19 Gouty deposits and necrosis of bone
20 Hypertrophy of suprarenal cortex
21 Hemorrhagic and sero-fibrinous polyserositis
22 Calcification about local injection sites

An examination of this pathological picture of lead poisoning indicates quite clearly that definite information about the action of lead can hardly be obtained from such a study The effect of lead might be considered too subtle to yield to this type of investigation. Certainly, many biological effects are evident which produce no apparent gross or microscopic lesions The conclusion must be drawn, therefore, that further knowledge of the action of lead on the organism can only be reached by physiological and chemical methods which deal with living rather than post mortem conditions

PART III PHYSIOLOGY

XI EFFECTS OF LEAD ON BLOOD CELLS

Changes in the blood are often the first and most important sign of lead poisoning in man These effects of lead are stippling and a secondary anemia. Although not an absolute indication of the

severity of the condition, the degree of the anemia and stippling of red corpuscles characteristic of plumbism usually runs parallel to the state of health These alterations in the blood doubtless contribute in some measure to the general debility, and therefore possess distinct clinical importance in diagnosis and prognosis Furthermore, the mechanism of their development throws light on many problems of biological interest

Stippling of red blood corpuscles Stippling of the red blood cells, or punctate basophilia, has long been recognized As early as 1885 Ehrlich (107) described this condition as the presence of basophilic granules in certain red cells Its association with lead poisoning was, however, first pointed out by Behrend (30) in 1899 The attention of many investigators was immediately focused on the study of stippling, its causation, its relationship to other basophilic conditions such as polychromatophilia and reticulation, and more especially its significance Because of the completeness of the recently published work of Pappenheim (351), and Naegeli (326), a detailed account of the results obtained and the theories evolved is unnecessary here, but it may be of value to point out some of the salient features of the problems involved and to suggest their significance This has been done by Key while working in this laboratory, and the following account of stippling is in the main his review of the subject

Three general questions as to the character of stippling must be considered before discussing its occurrence during lead poisoning (*a*) are the basophilic granules derived from nuclear or cytoplasmic substance, (*b*) are the changes caused by degeneration or regeneration, (*c*) are these granules related to other basophilic substances, polychromatophilia, and reticulum? After these problems have been solved, the action of lead in the causation of stippling may be studied as a specific example of a general process taking place in the blood

Origin, formation, and relationship of basophilic granules The derivation of basophilic granules in the red blood cells was one of the first problems studied

Ehrlich (107) believed stippling to be of cytoplasmic origin and was supported in his view by Smith (433) and Foa (145) Askanazy (13),

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however, in his earlier work thought that he could demonstrate all transitions between polychromatophilia, stippling, karyorrhexis, and karyolysis, and brought forth the theory that the granules of stippling are nuclear fragments This latter view was generally accepted until Grawitz (176) and his school demonstrated its invalidity by showing quite conclusively that the granules are of cytoplasmic origin This proof of the correctness of Ehrlich's theory is based on the complete independence of basophilic granules and nuclear substance Not only have true direct transitions between nuclear fragments and stippling never been observed, but the granules are frequently seen in corpuscles with intact nuclei and in erythroblasts during mitosis Furthermore, stippling occurs in blood containing no normoblasts (i e, during lead poisoning), but it is completely absent in the bone marrow where nucleated red cells are most abundant Grawitz finally pointed out that the granules have none of the characteristics of nuclear substance—they cannot be photographed with ultra-violet light, they are not stained with Ehrlich's tri-acid mixture, and they do take a red stain with Pappenheim's methyl green pyronin.

Since the cytoplasmic origin of these basophilic granules has been definitely established, the question of the nature of their production may be considered

That stippling is a regenerative process, was the view held by Engle (119) because of the appearance of stippled cells in the embryo He believed that the presence of basophilic granules in the adult signified a return to the embryonic type of blood formation and was supported in his opinion by Naegeli (326), Koenig (239), and Askanazy (14) Most workers now, however, support the view that stippling is produced by degeneration The absence of punctate basophilia in the bone marrow (482) (176) (351) (228) (14) (233) (234) as well as the even more significant fact that stippling is associated with pathological processes (176) (249) (30) (377) (398), renders its degenerative character quite probable

To understand clearly the nature of stippling, its relation to the various other basophilic substances of the cytoplasm must be studied Many evidences of the close alliance to the polychromatophilic substance may be found, and these have been summarized by Key as follows

The same fixatives are effective for both, and the same differential stains. Both are soluble in alkalies, and insoluble in water and various acids, both

contain potassium, but neither contains iron, using McCallum's (287) method for the detection of these elements, and both give a slightly positive Millon's test Finally, in a single preparation all transitions between polychromatophilic cells, stippled polychromatic and stippled orthochromatic cells may be demonstrated Reticulum also has the same staining and chemical characteristics Its relation to stippling may be demonstrated by drying smears incompletely and staining them in an isotonic medium In such preparations all stages between stippled fragments and typical reticulum may be observed By incomplete fixation with osmium tetroxide vapor all transitions from fragmentation to diffuse polychromatophilia (233) may be demonstrated Consequently the close relationship between stippling, reticulum and the polychromatophilic substance is apparent

The general significance of stippling All the facts thus far mentioned point strongly toward the view that stippling is a granular degeneration or coagulation of basophilic substance To throw more light on this question, Key (234) performed some interesting and convincing experiments which have an important bearing on the significance of stippling

He administered lead to two groups of rabbits—one normal and the other made anemic by previous intermittent bleeding In normal animals the marked reduction in the number of red blood cells which occurred promptly after giving lead was followed by a striking increase in the number of circulating young cells This indicates that the anemia was caused primarily not by suppression of bone marrow activity but by destruction of existing blood cells In the anemic group of animals the diminution in the red cell count after exposure to lead was much less than in those which were not bled In fact, in some of the anemic rabbits there was a transitory rise in the cell count above that obtained before lead was given This Key explained as due to a probable stimulation of bone marrow The nature of this stimulation is not definitely known but may depend on the fact that the frequent bleedings make constant demands upon the bone marrow and render it hyperactive In such a condition a transitory and slight destruction of blood by lead might call forth excess blood formation very rapidly Stippling appeared in the blood of both groups of animals within twenty-four hours after administration of lead and persisted for ten days to three weeks or more Simultaneous counts of the red, reticulated, and stippled cells demonstrated the very significant fact that the number of stippled

cells varies directly with the number of young cells and is much greater in anemic than in normal animals.

By these experiments on rabbits, Key practically proved that stippled cells are young red corpuscles which are exposed to lead and that the granules are probably aggregations of part of the basophilic substance into small discrete masses This reaction is probably degenerative in its nature That stippling is not an artefact produced by staining, Key demonstrated by examining fresh blood under the microscope In this he found cells containing slightly refractile clear masses exhibiting Brownian movement The number of these corresponded roughly to the number of stippled cells present in stained preparations of the same blood He also thought that by a modification of the McJunkin (290) method of staining he could demonstrate that stippling is not precipitated lead within the cell because many stippled cells contained no black granules of lead sulphide and most of the cells containing these granules were not stippled Moreover, both stippling and precipitated lead may occasionally be seen within a single cell

This method consists of fixing smears of blood in a mixture of alcohol and ether for twenty-four hours and exposing them for thirty minutes to a 10 per cent NH_4OH solution saturated with H_2S They are then counterstained with eosin or, better still, pyronin It must be emphasized, however, that the amount of lead in blood is so very small that the actual interpretation of black particles as lead sulphide must be made with very great caution, if at all

This rather detailed discussion of the derivation of the granules in stippled red blood cells is pertinent here because it emphasizes the pathological nature of stippling and its development as the result of a toxic action on the young cells Since stippled cells are never found in the bone marrow, even when they are very numerous in circulating blood, this toxic action apparently occurs peripherally.

Stippling as a clinical phenomenon Before discussing the mechanism of the production of stippling by lead, it is essential to point out that the presence of punctate basophilia in the blood is not a specific sign of lead poisoning

It occurs in pneumonia in infants (58), in pernicious anemia, leucemia, pseudoleucemia, in the anemias due to cachexia of neoplasms, and in the anemias caused by hemorrhage when the hemoglobin is retained in the organism (176) Stippling also may be seen in the blood of embryos of mice (118), rabbits, guinea pigs, sheep and swine (326), but is rarely found in human embryos (239) Hamilton (195) reports that it has been observed in cases of aniline poisoning Thus, stippling is not caused by the action of lead alone, but may be produced by other toxins and perhaps during normal embryological conditions This suggests that stippling represents a general pathological reaction of the cell rather than some specific phenomenon

But the occurrence of stippling in the red cells in these various other conditions is relatively rare and slight as compared with the frequency and intensity of its appearance during plumbism For this reason the demonstration of stippled red cells in the blood has come to be considered an almost definite evidence of absorption of lead Many clinicians even go further than this and consider stippling the most important sign for the diagnosis of lead poisoning (See section XVII)

Methods of staining stippled cells The choice of methods for staining red blood cells to demonstrate stippling is of importance, because unsuitable or poorly applied stains may fail entirely to show basophilic granulation

Ordinary differential stains such as Wright's, Jenner's, and Hastings may not bring out the basophilic granules even when there are many stippled cells Over-staining with these so that the effect of methylene blue is pronounced usually demonstrates the granules Therefore, to obtain good pictures of punctate basophilia with these stains, the smears must have a distinctly blue tinge so that the neutrophilic granules stand out clearly Hayhurst (204) prefers Skelton's or Harlow's method of staining a dried blood smear with eosin (1 per cent absolute methyl alcohol solution) for one minute, then with methylene blue (1 per cent absolute methyl alcohol solution) for one minute, and washing free with water Schwarz (407) recommends the examination of a thick blood smear for a rapid diagnosis, and Seiffert (415) suggests staining the unfixed smear with Loeffler's methylene blue This latter method is very objectionable, according to Engel (118), because unfixed smears stained with methylene

blue show many granules in normal cells Seiffert (416), however, believes that real stippling can easily be differentiated from these false granules. These "pseudo" granules are pale, rather irregular, and are usually seen in a network of reticulum, while true granular basophilia is very dark and may be intensified in the fixed preparation To be significant in the diagnosis of lead poisoning Seiffert holds, true stippled erythrocytes must be found in every five or six fields. Schreiber (405) uses fresh preparations of blood which are stained with a complex "combined brilliant cresyl blue Giemsa dye," and Sellers (417) mixes a drop of fresh blood with a drop of 1 in 500 methylene blue on a slide In our work the best results in demonstrating the granules were obtained with Unna's alkaline methylene blue solution, which has been employed by many investigators in this field It consists of one part methylene blue and one part potassium carbonate per hundred parts of water, and is used as follows Dried thin blood smears, which have been fixed with methyl alcohol and dried, are flooded with a 1·15 dilution of the stain and allowed to stand for fifteen minutes The stain is then washed off with water until only a faint bluish green tinge remains When prepared in this manner the red corpuscles appear light greenish blue, the nucleus of leucocytes and the basophilic granules dark blue The stippled cells usually are somewhat polychromatic

The general biological problems related to stippling As this survey shows, some of the fundamental problems of stippling are still unsolved, and several questions of both biological and practical significance merit further investigation For instance, basophilic granules are considered to be derived by some degenerative process from the basophilic substance of young corpuscles which normally gives the cells their polychromatophilic appearance But lead has never been known to produce stippling *in vitro* (393) (439) (234) Another suggestive problem is the apparent restriction of stippling to certain species In man, rabbits, guinea pigs, and rats, lead poisoning is characterized by the appearance of basophilic granules in the red blood corpuscles, but in the blood of cats, chickens, pigeons or amphibia stippling has never been produced (234) (303) (196) (328) in spite of the fact that the corpuscles of amphibia and birds are known to contain basophilic substance. White and Pepper (491) report an atypical stippling in dogs poisoned with lead The granules, however, were brought out after heating the smears and were clumped and large, quite unlike the usual picture Also the fact that they

describe stippling in bone marrow cells, which has not been obtained by other workers, makes their finding of stippling in dog's blood very questionable In our work innumerable examinations of rabbit's (234), rat's and man's blood have demonstrated without question the occurrence of punctate basophilia in these species but distinct stippling has never been seen in the blood of cats even when symptoms of plumbism are very marked It is true that occasionally a suggestive mottling appeared but certainly no definite stippling The broader significance of this will be emphasized later in a discussion of the relation of stippling to other blood changes caused by lead, but it is sufficient here to indicate that failure to produce stippling *in vitro* suggests that this is not a simple phenomenon Furthermore, the occurrence of stippling in some animals and its absence in others implies a biological and perhaps chemical variation between red blood cells of different species

Anemia The other important change in the blood caused by lead—the anemia—has long been recognized and repeatedly demonstrated As early as 1831 Laennec (244) described pallor of tissues and thinness of blood in cases of plumbism at autopsy In 1840 Andral and Gavarret (8) first actually counted the number of red blood cells during lead poisoning and found the count much lower than normal Now an erythrocyte count is a routine clinical procedure in lead intoxication In fact, in some experimental work anemia is considered an index of the degree of lead poisoning in the animals A study of lead anemia is important not only because of its clinical significance, but also because a clear understanding of the action of lead on the red blood corpuscle, as an isolated cell, throws considerable light on the mechanism of its reaction with other body cells

This anemia has been attributed both to peripheral destruction of blood and to lesions in the bone marrow which result in insufficient production of blood cells

The latter explanation was supported by Raimondi (373) and by Stockman and Charteris (445) who described a preliminary hyperplasia of bone marrow followed by definite gelatinous degeneration Wolff (496) also believed that the bone marrow is affected but not in the early stages of plumbism, while Meillère (293), Schnitter (402), and Sellers (417) all stated that lead definitely injures both blood corpuscles and bone marrow These

are the only workers who thought that lead may interfere with blood f
tion. But, although bone marrow may possibly degenerate in adv
lead poisoning, the preponderance of evidence indicates clearly tha
anemia is probably caused by destruction of cells in the circulating bl
a fact first suggested independently by Heubel (213) and Bouchard
and later by Rauch (377) and others The evidence for this is quit
clusive. Early in lead intoxication there is hyperplasia of bone ma
and the large numbers of nucleated and reticulated red cells in circu
indicate that regeneration is taking place. Key (234) has shown t
rabbits within twenty-four hours after the ingestion of 1 gram of lea
number of circulating red cells decreases more than 20 per cent, bu
many nucleated and reticulated erythrocytes appear That hematop
in lead poisoning is not impaired can also be demonstrated by a retic
count as a measure of young cell formation This was 6 per cent a
per cent in two of our lead colic patients who had red counts of ap
mately 3,500,000 cells These figures indicate clearly that the
marrow was actively combating the anemia

Excretion of the decomposition products of the red blood
has also been studied.

As long ago as 1871 Heubel (213) obtained evidence of the direct a
of lead on blood by finding increased pigment in serum, bile and
Meillère (293) observed a definite increase in the urinary excretion o
which is largely derived from red corpuscles That the presence of he
porphyrin, a product of blood destruction, in urine is an important f
in diagnosing plumbism is a fact more commonly known in Europe th
this country. Brady (52), in this laboratory, demonstrated very ma
hematuria and an increase in the excretion of bile pigments from the
mon duct of rabbits poisoned with lead acetate This, however, was
in rather massive doses, 1 8 gram by stomach tube in some experiment
125 mgm intravenously in others, and represents a very acute poiso
In human cases, Jones (229), using the regular Lyons' method, ha
tained similar evidence of marked destruction of circulating blood.
found that the bile obtained by duodenal tube as well as the blood pl
from several patients suffering from lead poisoning contained much h
concentrations of bile pigment than that from normal subjects All
results point to the probability, therefore, that lead acts on the perip
blood and ordinarily has no primary inhibitory effect on hematopoiesi

Experimental Observations. Since lead seems to affect dir

the corpuscles in the circulating blood, it is of prime importance to understand the exact chemical and physiological nature of the reaction For the last three years therefore, we have studied this problem (15) (16) (17), and many *in vitro* and *in vivo* experiments were performed which afforded an explanation of the anemia of lead poisoning

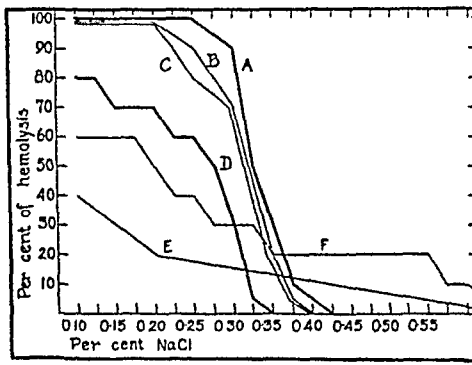

FIG 24 SODIUM CHLORIDE HEMOLYSIS THE EFFECT OF VARYING THE QUANTITY OF LEAD

The curves show the effect on hemolysis of previous exposure for one hour of washed red blood cells to

B, 1 cc of red blood cells exposed to 0 002 mgm of Pb as chloride
C, 1 cc of red blood cells exposed to 0 004 mgm of Pb as chloride
D, 1 cc of red blood cells exposed to 0 01 mgm of Pb as chloride
E, 1 cc of red blood cells exposed to 0 03 mgm of Pb as chloride
F, 1 cc of red blood cells exposed to 0 08 mgm of Pb as chloride

A shows the action of the control normal red blood cells in Ringer solution This chart indicates the per cent of hemolysis in each concentration of salt solution

Hemolysis in hypotonic saline solution One of the most striking phenomena observed in this work is the increased resistance of red blood cells to hypotonic saline solutions after exposure to lead.

As long ago as 1873 Malassez (275) noticed not only that anemia was a symptom of lead poisoning but that the blood cells were larger and thicker than normal Agasse-Lafont and Heim (2) have found slightly increased globular resistance in cases of plumbism, and von Liebermann (266), Orbán (343), and Hayhurst (205) have confirmed this observation In an

elaborate investigation Fici (135) has recently demonstrated the phenomenon *in vitro*. He also found that lead acetate not only increases the resistance of some cells to hypotonic saline solution but makes other corpuscles more fragile and suggested that these phenomena are due to the direct action of lead on the cell His work is very interesting in the light of our experiments.

In the experiments in this laboratory, washed human corpuscles from defibrinated blood were used and portions of these were exposed to lead as lead chloride in Ringer solution, the rest being treated with regular Ringer solution as controls. The pH of all solutions was kept at 6.5 and all manipulations were so standardized that they did not vary from day to day In every experiment the control tests allowed direct comparisons—a method which gives more accurate results than would comparisons with an average normal The so-called "fragility" or hemolysis tests to hypotonic saline were performed on the "leaded" and control cells Using this method, we found that exposure to a concentration of only two parts of lead, by weight, per million (1 cc. of corpuscles per 0 001 mgm lead) definitely increased the resistance of cells, while a solution of one part per hundred, thousand (1 cc of corpuscles per 0 01 mgm lead) produced so marked an effect that many of the corpuscles did not hemolyze completely in 0 1 per cent saline Normal cells, on the other hand, usually are completely hemolyzed by 0 25 per cent salt solution This result is illustrated by figure 24. It is striking that in the three tubes corresponding to those in which normal cells were first destroyed, there was no hemolysis of the least resistant "leaded" corpuscles If greater concentrations of lead were used, two phenomena at once became evident The resistance to hemolysis increased but some cells were so injured that they hemolyzed upon standing even in normal Ringer solution, although the controls did not (fig 24) The action of lead is therefore double. (*a*) the cells exposed to lead withstand greater change in osmotic tension, but (*b*) they break up more rapidly than normal cells

If the results of a series of these experiments dealing with hemolysis of cells in hypotonic saline solution are plotted (fig 25) so that each point represents the increase in per cent of hemolyzed cells, it appears clear that the cells are unevenly "leaded" or that two antagonistic

reactions are involved, one of which permits hemolysis in salt solution at concentrations above 0 225 per cent, while the other prevents hemolysis until the concentration falls to 0 1 per cent The invariable movement toward the left of curves representing hemolysis of "leaded" corpuscles suggests that the normal cells which hemolyze most easily are those most susceptible to lead and are rendered more

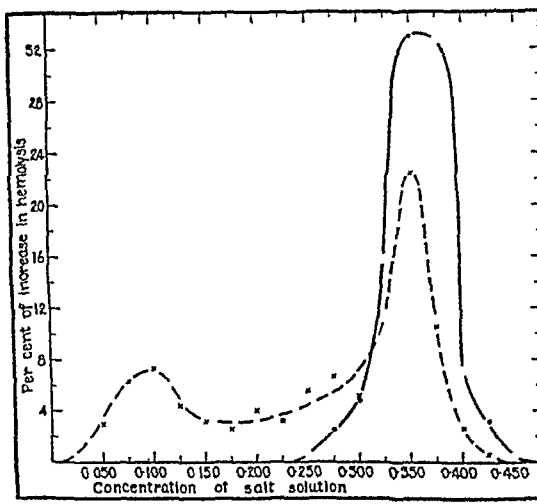

FIG 25 THE DISTRIBUTION OF HEMOLYSIS THE AVERAGE OF TEN EXPERIMENTS WITH CELLS EXPOSED TO 0 01 MGM OF LEAD AS LEAD CHLORIDE PER CUBIC CENTIMETER OF WASHED RED BLOOD CELLS FOR ONE HOUR

Each point represents the per cent increase of hemolysis over that in the next tube of greater saline concentration

●——— ● normal cells x - - - - - - x "leaded" cells

resistant by its action Which cells these may be—reticulated or mature, young or old—has not yet been definitely demonstrated

Attempts to reverse the effect of lead in changing the hemolysis of cells in hypotonic saline solution by "de-leading" them have proved unsuccessful and therefore suggest that the lead reaction is irreversible Moreover, our experiments show that the salts of other metals (cerium, aluminium, calcium, arsenic, cadmium, copper,

mercury) or substances like formalin and tannic acid have no comparable effect; whereas the other soluble lead salts, the nitrate and acetate, have the same effect as lead chloride. It is, therefore, probable that the action of lead on the resistance of red blood cells to hypotonic salt solution is an isolated phenomenon, at least in its intensity under these conditions Furthermore, this modification of the fragility of "leaded" cells in hypotonic saline solution affords a delicate method for the determination of the biological effects and chemical reactions of lead This reaction may be obtained in whole blood but more lead must be added than is necessary with washed red cells

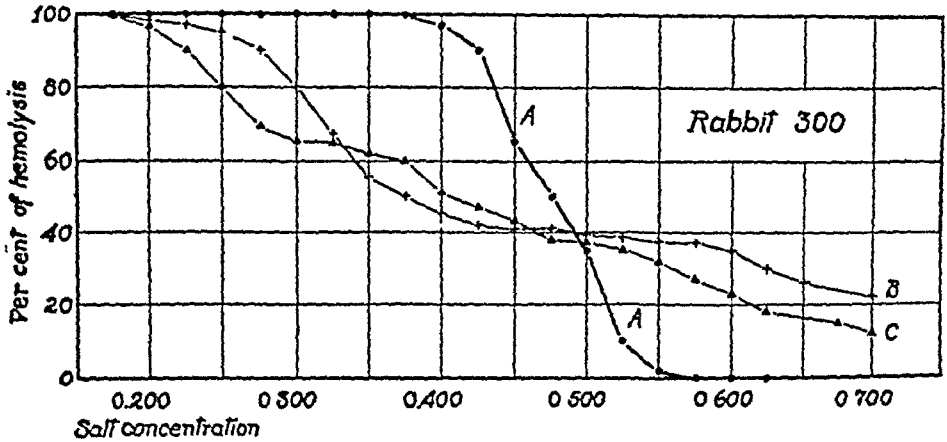

FIG. 26 SODIUM CHLORIDE HEMOLYSIS THE EFFECT OF ACUTE LEAD POISONING ON RED BLOOD CELLS IN VIVO

The curves represent the action of red blood cells washed in Ringer solution
A, before lead was given
B, nineteen hours after 1 gram of lead acetate was given by mouth
C, forty-three hours after 1 gram of lead acetate was given by mouth

If this reaction between lead and red corpuscles has any great significance its occurrence in life must of course be demonstrable. That it is, has already been shown in man by von Liebermann (266), Orbân (343), and Hayhurst (205) who observed that corpuscular resistance increases during acute lead poisoning Further investigations of the resistance of corpuscles have been carried out on rabbits Figure 26 shows the results obtained, and demonstrates that *in vivo* the corpuscles are affected just as *in vitro* Apparently many "leaded" cells resist the strain of hypotonic saline better than the control

corpuscles but some hemolyze quickly even in normal saline solution This justifies the assumption that the phenomena studied here *in vitro* are similar to those which may occur in life and are most probably related to development of the anemia of lead poisoning

Further evidence of connection between the modification of the hypotonic saline curve and the changes caused by lead *in vivo* is

TABLE 33

The parallelism between blood manifestations in various animal species

The effect of lead *in vivo* on the appearance of anemia and stippling in blood, and *in vitro*, on the hemolysis in hypotonic salt solution

SPECIES	DISTORTION OF HYPOTONIC SALINE CURVE TO LEFT FOLLOWING EXPOSURE TO LEAD*	APPEARANCE OF ANEMIA IN LEADED ANIMALS†	APPEARANCE OF STIPPLING IN LEADED ANIMALS†
Man	++	+	+
Rabbit	++	+	+
Rat	+++	+	+
Guinea pig	+	+‡	+‡
Dog	Negative	?	?§
Cat	Negative	Negative until just before death	No clear cut stippling Rare mottling of cells
Horse	Negative	?	?
Chicken	Negative	?	Negative ‖

* All exposed to same concentration, *i e*, 0 01 mg of Pb per cubic centimeter of washed red blood cells Plus means that the curve of "leaded" cells indicated distinctly increased resistance to hemolysis in dilute saline solutions

† Concentrations of Pb per kilogram of animal not constant

‡ Data from Sabrazès, Bourret, and Léger (393), and from Beresina and Engling (32a)

§ Stated by White and Pepper (491) to be positive, though not typical This observation has not been repeated

‖ Data from Key (234) and also from Meyer and Speroni (303)

indicated by the fact that the action of lead on hemolysis in hypotonic solution seems to run parallel to the appearance of stippling and anemia in different species of animals Table 33 shows that these phenomena have not been observed in all species but that in those studied all are either present or entirely lacking Thus, in man, rabbits, guinea pigs, and rats, stippling and anemia are easily produced and there is the characteristic change in the resistance to hemolysis in hypotonic salt solution after "leading" The red cells of horses,

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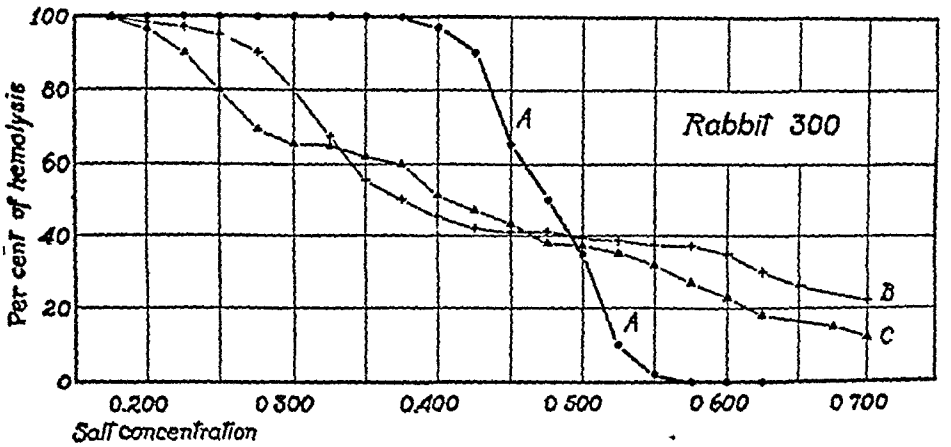

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Man	++	+	—
Rabbit	++	—	+
Rat	+++	—	+
Guinea pig	+	—‡	+‡
Dog	Negative	?	?§
Cat	Negative	Negative until just before death	No clear cut stippling Rare mottling of cells
Horse	Negative	?	?
Chicken	Negative	?	Negative‖

* All exposed to same concentration, i.e., 0.01 mg. of Pb per cubic centimeter of washed red blood cells Plus means that the curve of "leaded" cells indicated distinctly increased resistance to hemolysis in dilute saline solutions

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dogs, and cats, on the other hand, show no changes in resistance as the result of adding lead *in vitro* The effect of lead on the blood of horses had not been studied *in vivo* but the cat shows no signs of anemia and no definite stippling has ever been observed in its blood In the case of the dog, the occurrence of anemia has never been reported and the presence of atypical punctate basophilia in this animal rests on the work of White and Pepper (491). Tests to determine whether lead does react with cat's blood without apparent physiological effect were carried out by mixing a known quantity of lead with a given volume of cat's washed red blood cells The mixture was then centrifuged and the supernatant fluid treated with fresh human corpuscles Hypotonic saline tests were performed and were entirely negative for lead effect These experiments, therefore, show that the lead reacts with the corpuscles even though their resistance to hypotonic salt solution is not affected

It may be well to consider these results and their general physiological significance more fully. Three apparently distinct phenomena are involved—stippling, anemia, and changes in resistance to hypotonic saline solutions—yet the occurrence of all in certain animals and the complete absence in others points to some interrelationship Probably all three depend on one underlying cause Certainly this correlation between the hypotonic saline test and the other blood phenomena which occur *in vivo* points to the fact that this modification in the hemolysis of "leaded" cells in hypotonic saline represents a real physiological reaction and not an accidental *in vitro* change The variations in the properties of cells of different species found in these experiments suggest many possibilities and offer an attractive field for further investigation

Chemistry The action of lead on the red blood cell is probably chemical in its nature, and, as has been pointed out, the hypotonic saline test affords an excellent means of studying such a reaction Lead can no longer change the resistance of red blood cells to hypotonic saline solution if mixed with a small amount of blood serum before being added to the corpuscles In other words, some substance in the serum so reacts with lead as to prevent combination with red blood cells subsequently added Using this finding as a basis, each of the known constituents of red blood cells (sodium

bicarbonate, inorganic phosphate, lecithin, cholesterol, euglobulin, pseudoglobulin, hemoglobin, and albumin) in pure form and in an amount equivalent to that in a given quantity of blood was mixed with lead After allowing time for reaction between the lead and the blood constituent, red cells were added to the mixture and the usual hypotonic saline test was carried out A normal curve indicated that the blood constituent had obviously so combined with lead during the preliminary treatment as to prevent the action of lead on the blood cells On the other hand, if the curve was that characteristic of "leaded" cells there had been no interaction between the substance tested and the lead In all experiments, the solution of blood constituents simulated as far as possible the conditions in the blood, especially with respect to hydrogen ion concentration and osmotic pressure The exact constituents of the blood cell are not definitely known, certainly not their quantitative distribution In fact, the presence in the corpuscle of some of the substances mentioned has been questioned But in all this work the generally held opinion of the chemical nature of the red blood cell has been accepted Results of these tests show definitely that inorganic phosphate, in the concentration normally found in serum or corpuscles, neutralizes the same quantity of lead as does whole serum This can, therefore, completely account for the action of lead on red blood cells Sodium bicarbonate also neutralizes lead, but ten times as much as occurs in an equivalent neutralizing quantity of serum is required None of the other constituents tested under these conditions combined with lead The failure of lecithin to unite with lead, as well as negative results with sodium glycerophosphate, indicates that such organic or bound phosphate plays no direct part in the action of lead

There is still further evidence that lead unites with the inorganic phosphate of the blood Serum with increased inorganic phosphate content, obtained from nephritic patients, neutralizes the effect of lead to a high degree Moreover, when inorganic phosphate diffuses from red blood cells into the surrounding Ringer solution lead no longer acts upon the corpuscles because of its neutralization by the diffusate If these cells are washed free from the diffusate, however, lead can again react with them These phenomena indicate that the action of lead upon red blood corpuscles can be entirely explained

by the union of lead with the inorganic phosphate of the cell It is important to point out here that when a lead salt unites with phosphate the very insoluble $Pb_3(PO_4)_2$ is formed with the liberation of free acid

$$3PbCl_2 + 2Na_2HPO_4 \rightarrow Pb_3(PO_4)_2 + 4NaCl + 2HCl$$

Fragility With this chemical picture in mind it is of interest to consider some of the physiological changes which occur when lead acts on corpuscles The physiological action of lead on the red blood cell is very striking If red corpuscles, treated with a lead salt, are permitted to stand in Ringer solution, an abnormally large number of these "leaded" cells hemolyzes within a few hours This demonstrates that lead shortens the life of the cell The increased fragility after exposure to lead, when cells are rotated or treated with carbon dioxide, is even more marked and is evidence of the fact that slight trauma can destroy "leaded" cells very easily. Rous and Robertson (391) and recently Broun (57) have attributed destruction of blood cells to mechanical trauma, and these experiments illustrate that cells injured by lead are very much more fragile than normal, and therefore far more susceptible to the trauma of circulation This is probably a very important factor in the increased destruction of blood in plumbism.

Specific hemolysis Anti-human serum, obtained from a rabbit immunized against human blood, caused much more rapid and complete hemolysis of "leaded" cells than of control cells The significance of this is not yet clear, but may have some bearing on the explanation of mechanisms of various reactions which occur in hemolysis

Agglutination One of the important practical problems connected with blood is the agglutination of corpuscles by heterologous sera Experiments performed on human blood with Group 2 or Group 3 corpuscles and Group 4 serum consistently showed that after exposure to lead agglutination is slow and slight, if it occurs at all, whereas in the control tubes exposed merely to Ringer solution strong agglutination follows the addition of serum (fig 27).

Stickiness of red blood corpuscles The discovery that lead inhibits agglutination of red blood cells in heterologous serum naturally raises

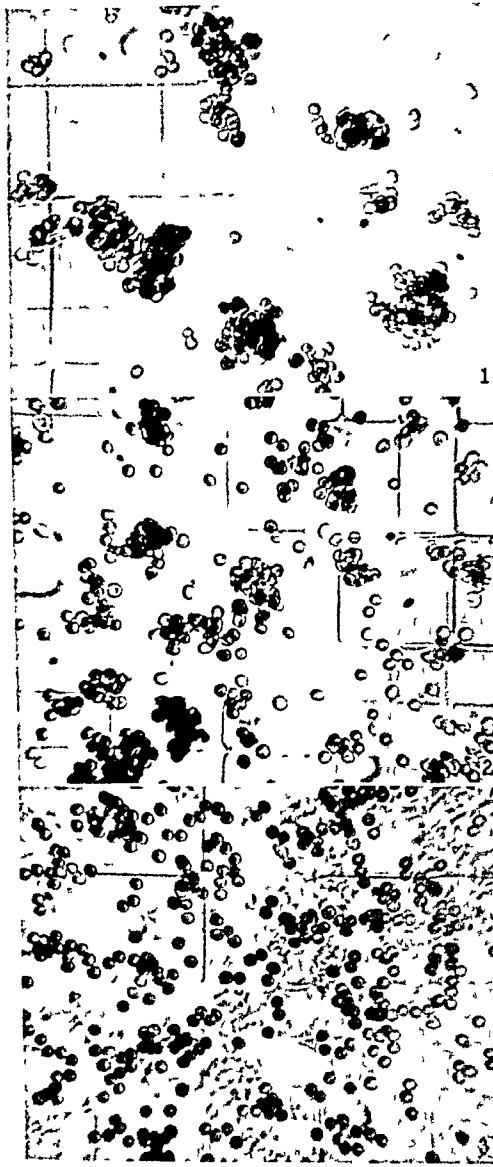

Fig. 27. Effect of Lead on the Accumulation of Group II Cells by Group IV Serum.

the question of whether it affects the natural stickiness of the cell surface or whether the addition of agglutinating serum causes the change This problem was studied by means of Fenn's (133) method which consists in placing a suspension of cells in a glass chamber which is rotated 180° after a given interval The number of cells which remains on the ceiling of the chamber, as well as the number which falls off, is then determined under the microscope It was found that normal control cells remained on the ceiling of the chamber after rotation, whereas a large proportion of "leaded" cells fell From this the conclusion may be drawn that after exposure to lead the surface of red blood corpuscles is less sticky than normal There are three possible explanations for this In the first place the lead may have added weight to the cell This is hardly probable because of the very small amounts used However, the effect of the lead is actually to increase the specific gravity of the corpuscle due to a marked shrinking (see page 148) But this increase in weight is probably not the important factor in decreasing the stickiness of "leaded" cells because the same phenomenon is illustrated in the changes with iso-agglutinins where variations in specific gravity are of no significance Furthermore, this difference in stickiness between "leaded" and normal cells does not occur unless serum is added to the media If the variation was merely due to changes in weight, it should be seen in any medium The other possibilities which must be considered as causing this loss in stickiness are that the charge of the cell surface may be changed (see page 149), or that because of alteration in its chemical composition, the surface may have changed its physical characteristics (see page 151)

In the case of polymorphonuclear leucocytes, obtained from the peritoneal cavity of rats after aleuronat injections, no definite difference between the stickiness of "leaded" cells and that of normal controls could be observed It therefore seems probable that lead affects the surface of white and red cells differently

Phagocytosis This finding is especially interesting in view of the work of Fine (137)

He studied the phagocytizing ability of rat's white blood cells for particles of metallic lead, basic lead carbonate, lead chromate, and lead sulphide,

using the "film method" of Fenn (132) The number of particles ingested by the leucocytes is determined and thus the rate of phagocytosis Fine found that metallic lead is less readily phagocytozed than the lead salts and suggests the possibility of inhaled metallic lead acting as a foreign body in the lung and exciting fibrosis

These results brought up the question of the possible effect of soluble lead on the phagocytozing power of the leucocytes To study this the leucocytes were exposed to varying concentrations of lead chloride and the rate of phagocytosis for basic lead carbonate particles was determined Although only two experiments were carried out in which the cells were subjected to the lead chloride for more than twenty minutes, these show very definitely that lead chloride in as low a concentration as 0 02 mgm of lead per cubic centimeter of suspension is able to retard the ingestion of particles very markedly, which demonstrates rather definitely that soluble lead injures the leucocyte

The problem that these results bring up suggests many implications which cannot be discussed here Of particular interest, however, is the failure of lead to affect the surface of the white blood cell, indicated by the stickiness experiments, and its inhibiting action on their phagocytozing power Of course it must be remembered that the results of the stickiness experiments on white cells is only negative evidence and may simply mean that a change in the surface could not be detected by this method The conclusion seems evident, therefore, that either variation in stickiness is not an absolute indication of surface changes, or that lead can interfere with certain functions of the white blood cell and leave its surface unimpaired

Permeability of the cell surface The salt hemolysis test is based upon the permeability of the cell to water, and the elasticity of its surface When osmotic tensions of surrounding fluids vary, the cell swells or shrinks because of changes in water content, and only its elasticity determines the degree of change of osmotic pressure which it can withstand without hemolysis Since the cell responds thus, by changing its volume, the specific gravity of normal and "leaded" cells must be an index of their permeability to water and therefore of changes in elasticity Using the benzyl benzoate-oil method

(384)[2] it was found that "leaded" red blood cells always have distinctly higher specific gravity than normal cells. The specifi gravity of normal red blood cells varies between 1 088 and 1 09((average 1.091), that of "leaded" cells between 1 104 and 1 11: (average 1 108), a difference sufficiently great to be well outside

TABLE 34

The specific gravity of red blood cells after exposure to lead for varying lengths of time

TIME	SPECIFIC GRAVITY
15 minutes	1 089
30 minutes	1 098
1 hour	1 115
2 hours	1 113
3 hours	1 112

TABLE 35

Effect of osmotic changes on specific gravity

CONCENTRATION OF RINGER SOLUTION		SPECIFIC GRAVITY OF		CHANGE OF SPECIFIC GRAVITY FROM CELLS IN NORMAL RINGER SOLUTION		TOTAL CHANGE OF SPECIFIC GRAVITY	
Before and during "leading"	After "leading"	Normal cells	"Leaded" cells	Normal cells	"Leaded" cells	Normal cells	"Leaded" cells
0 9	0 9	1 089	1 111				
0 9	0 5	1 065	1 103	−24	−8		
0 9	1 35	1 109	1 115	+20	+4		
0 5	0 9	1 092	1 113	+3	+2		
1 35	0 9	1 090	1 114	+1	+3		
0 9	0 9	1 089	1 106				
0 5	0 5	1 069	1 097	−20	−9		
1 35	1 35	1 104	1 110	+15	+4		
0 5	1 35	1 107	1 115	+18	+9	+38	+18
1 35	0 5	1 073	1 095	−16	−11	−31	−15

the limits of experimental error This increase in specific gravity, as would be expected, develops slowly and becomes maximal only

[2] This method depends upon the suspension of a small drop of corpuscular cream, centrifuged free from serum, in mixtures of benzyl benzoate and cotton-seed oil By varying the proportions of these two fluids, media of known varying specific gravity are obtained The one in which the red blood cells neither rises nor sinks, but remains suspended, is equal to the specific gravity of the corpuscles

after an hour (table 34) Since this change may be observed after the addition of a quantity of lead as small as 0 01 mgm per billion cells, it cannot be explained by the mere increment of the heavy metal It must be due to an actual increase in the density of the cell caused by a decrease in its volume after exposure to lead although the osmotic tension of the surrounding fluid is not altered

When the osmotic tension of the fluid is varied and the specific gravity of the cells determined, very interesting differences between normal and "leaded" cells appear Figure 28 and table 35 show the result of placing normal and "leaded" cells in concentrations of 0 5, 0 9, and 1 3 per cent Ringer solution "Leaded" cells proved to be heavier in every case Their range of swelling and shrinking

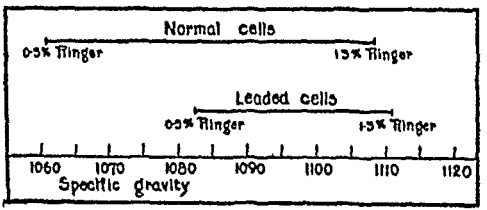

FIG 28 EXPERIMENT DEMONSTRATING TOTAL CHANGES OF SPECIFIC GRAVITY OF CELLS IN VARYING CONCENTRATIONS OF RINGER SOLUTION

is far less than normal, but they are not impervious to water for some variations in weight may be observed and the usual specific gravity is regained in 0 9 per cent Ringer solution even after exposure to solutions of other concentrations The difference observed, therefore, is apparently due to the fact that the exchange of substances through the cellular membrane is restricted—decreased permeability to water The most reasonable explanation for this phenomenon is that the cell surface probably shrinks after exposure to lead and becomes less elastic or that the charge of the cell surface is so changed as to repel a definite quantity of water

A second method used to study the difference in swelling capacity between normal and "leaded" cells was to determine the volume of a unit number of corpuscles of each kind This was based on the assumption that the number of cells in a unit volume would vary with

the size of the cell Normal corpuscles and those exposed to lead were changed from a 0 9 to a 0 6 per cent medium From the red cell counts and hematocrit determinations of the control and "leaded" cells in both 0 9 and 0 6 per cent Ringer solutions, the number of corpuscles packed into a unit volume in each case could be obtained and thus the change in cell volume The results from such experiments cannot be considered more than a check on the more exact specific gravity experiments because of the increasing error involved in cell counts as blood is diluted However, in two cases in which the theoretical values closely approximated those obtained it was found that normal cells could swell 19 per cent in one experiment and 28 4 per cent in the other when changed from a 0 6 to a 0 9 per cent medium The "leaded" cells in the first case could swell only 0 06 per cent and in the other 6.6 per cent Thus, the inability of "leaded" cells to swell, except within narrow limits, is apparent

The appearance of the cell The specific gravity experiment suggests that microscopic examination of the corpuscles might demonstrate a change in size after exposure to lead That this is true was shown by measuring large numbers of cells under an oil immersion lens with a micrometer eyepiece When corpuscles are exposed to lead, the cell margin first becomes pale and then swelling occurs Within an hour this is followed by shrinkage and a definite crenation of the surface Then gradually the irregularities of these shrunken cells disappear and the outline becomes smooth and quite refractile to light Maurel (285) also found that lead causes a marked deformity of cells These phenomena, as well as others which also vary with time, suggest that the corpuscles react slowly with lead and that, under the temperature conditions of these experiments, equilibrium is established only after from two to five hours

The interior of the cell Since all these experiments have shown that lead affects the surface of the cell, the question arises whether there is any involvement of the interior of the corpuscle That hemoglobin does not react with lead has already been pointed out. This is further corroborated by experiments carried out with Dr. A. V. Bock at the Massachusetts General Hospital, which showed that the gas exchange of "leaded" cells was apparently normal That lead can penetrate the corpuscle and react with other substances

cannot be ruled out absolutely However, the chemical findings which have been described previously indicate that the only cell constituent of those investigated in these experiments with which lead could unite is the inorganic phosphate It could not combine with the organic phosphates, such as lecithin, unless they broke down and liberated phosphate in a simple state Moreover, it is apparent that the first inorganic phosphate with which lead would come in contact would be that nearest the surface

The explanation of the effect of lead How can the changes in red blood cells caused by lead be best explained? Apparently they all depend upon the union of lead with inorganic phosphate and are largely surface phenomena But diffusion experiments prove that the mere removal of ionized phosphate by lead is hardly sufficient cause for these effects Despite the loss of phosphate by diffusion from red blood cells which stand in Ringer solution for several hours, such cells show no variation of the hypotonic saline curve from normal The possibility that lead phosphate precipitates on the surface of the cell and exerts a modifying influence there must be considered Phosphate probably unites with lead to form $Pb_3(PO_4)_2$ This may be precipitated in the membrane where its very presence may alter the colloidal properties That it forms a complete coating on the outside of the cell, however, is hardly probable because of the very small amount of lead used The possible surface area involved in this reaction cannot be determined because all cells are not equally affected by lead, and therefore calculations of the amount of lead available for each cell or of the amount of phosphate involved per unit of cell surface are not justified A rough approximation, however, may be made which shows that the area of a red cell is at least of the order of magnitude of 10^{-7} sq cm , while that of the lead molecules available for that cell is at most of the order of 10^{-12} sq cm Such a great difference makes the possibility of a superficial coating of the cells, even by a single molecular layer, highly improbable Changes in the charge of the cell surface might explain the action of lead This is suggested by phenomena like the disappearance of agglutination and the loss of stickiness and by rather marked differences between the agglutination of normal and "leaded" blood cells observed when tested with colloidal iron and arsenic Macroscopic cata-

phoresis experiments however, showed no differences in migration between "leaded" and control cells

The probable explanation of the effect of lead may rest on another basis It has been shown that when lead chloride interacts with inorganic phosphate, free acid is liberated This might be of importance in explaining the action of lead salts on red blood cells Although buffer action would prevent any appreciable change in the hydrogen ion concentration of the blood as a whole, there might be a marked local temporary change at the surface of the cells The objection may be raised that normally the buffers of the blood are quite adequate to take care of any acid produced But it must be remembered that we are dealing here with a cell surface capable of local reactions and variations in permeability Furthermore, the principal buffer, the phosphate, interacts with lead to form insoluble phosphate and free acid, so that its buffer action is diminished

A series of experiments with collodion sacs illustrates this When equal volumes of Ringer solution containing lead chloride (0 1 mgm lead per cubic centimeter) and serum (diluted three times with Ringer solution) are mixed, no macroscopic precipitate forms If, however, the serum alone is placed in a collodion sac and this is then suspended in the lead solution, a heavy precipitate forms at the membrane If methyl red is added to each of these solutions, both remain alkaline to the indicator, but the membrane itself turns red. This demonstrates that acid forms locally The same result is obtained if phosphate solution is substituted for serum.

These experiments with the collodion cells and the chemical reactions involved, therefore, indicate that the two reactions of lead on blood cells are precipitation of insoluble lead phosphate and production of acid These local reactions may not be the only causes of colloidal change but are very important factors in producing all the phenomena described, for anything which changes the colloidal state of the cell surface probably affects its properties

Summary. All the observations which have been presented fit into a complete and satisfactory explanation of the action of lead on blood. *In vitro* the exposure to a very small amount of lead greatly alters the surface of the red blood cells and causes them to shrink Their consequent relative impermeability to water renders

them incapable of swelling as much as normal cells This enables such cells to withstand a marked increase in their resistance to different osmotic surroundings, evidence for which is found in the abnormally small degree of hemolysis in salt solutions of very low concentration This suggests that "leaded" cells should be really stronger and more durable than normal cells But this is not the case On the contrary, "leaded" cells are relatively short-lived and hemolyze readily as the result of slight trauma Experiments with rabbits suffering from acute lead poisoning show that these phenomena also occur *in vivo* In addition to causing these changes in permeability, lead also alters the physical properties of red blood cells so that they lose their normal stickiness and are no longer agglutinated by sera of the different iso-agglutinating groups All these changes are evidence of surface alterations in the cell The interior of the cell is not affected, i e, the physiological properties of the hemoglobin remain normal The chemical reactions which cause the physical changes in the cell are precipitation of insoluble lead phosphate and formation of acid These cause the "leaded" red blood cell to change from an elastic, distensible sac to one which is contracted, relatively inelastic, and brittle In such a condition the cell can so poorly withstand the trauma involved in circulation that this lack of resistance probably explains the marked destruction of peripheral blood in lead poisoning Because of the loss of circulating red cells in this way, there is a compensatory regeneration of erythrocytes Many of these young cells are affected by lead after they enter the circulation so that their basophilic substance is coagulated or clumped together and they give the characteristic granular appearance known as stippling *In vitro* experiments indicate that lead may also have some inhibiting effect on the phagocytozing ability of polymorphonuclear leucocytes

XII EFFECT OF LEAD ON THE GERM CELLS

The effect of lead on the red blood cells may well be merely a striking example of an action which takes place in other cells of the body The literature indicates that the germ cells are affected, but the data are generalized and only demonstrate that when either parent is subjected to the action of lead, the development of the young is impaired

[illegible] was the first to notice this In 1860 he reported [illegible] the mother or even of the father often caused still- [illegible] infant mortality of children born of such parents was [illegible] during the first three years of life This statement is [illegible] ation of clinical cases, one of the most noteworthy of [illegible] woman who bore three normal children before her exposure [illegible] exposure she became pregnant ten times, but the only child [illegible] died at the age of five In the same year Mattei (284) [illegible] striking case Others (25) (409) (367) (95) have since [illegible] to these reports by giving evidence that, after the [illegible] stillbirths and miscarriages are frequent. Verhaeghe [illegible] obtained from 90 lead workers, to whom 487 children [illegible] exposure Although some of these workers had had [illegible] before exposure, 107 of those conceived after absorption [illegible] dead at birth, and 93 died before reaching two years of age [illegible] (361) reported the results of experiments performed with [illegible] pigs during experimental lead poisoning He found that [illegible] abortion rarely occurred, many of the young were born dead or [illegible] after birth. Upon examination, lead proved to be present in their [illegible] a comparatively long time after birth In spite of these re- [illegible] however, clinical data seem to demonstrate that le[illegible] especially in [illegible] of diachylon ointment (376) (411) (184) (1[illegible] [illegible] course, dangerous abortifacient.

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[illegible] one case, Oui (348) [illegible] ths o[illegible] [illegible] mother was suffering fr[illegible] in [illegible] frequently had ab[illegible] d [illegible] a healthy child by an[illegible]

Lewin (260) states t[illegible] in man when either [illegible] causes, the deleterious eff[illegible]

Goadby (252) confirms [illegible] on guinea pigs and also [illegible] Further very careful inv[illegible] (487). He poisoned both [illegible] mating and found that wh[illegible]

TABLE 36

Analysis of the tissues of offspring for stored lead after administration of lead acetate to mother

ANIMAL	DATES OF ADMINISTRATION OF LEAD	AMOUNT OF LEAD ACETATE GIVEN	DATE OF DELIVERY	NUMBER OF OFF SPRING	WEIGHT OF MATERIAL ANALYZED	LEAD FOUND	REMARKS
		grams			*grams*	*mgm*	
Rabbit 294	November 14	0 581	December 17	8	50	0	Killed directly after birth
	November 28	0 581			40	0	
	December 10	0 830					
	December 14	0 830					
		2 822					
Cat 337	October 5 to March 1	7 7	September 14	4	70	0	Killed directly after birth
					255	0	At end of two weeks Mothers milk the only food All kittens died
Rabbit 300	January 2	1 0	Animal died March 4		91 6	0 54	Uterus plus fetuses analyzed
	March 1	1 0					

nitely underweight and weak The "leaded" males were not usually sterile, but when mated with a normal female the weight of their young was 18 5 per cent below normal If the female was "leaded" and the male normal, the reduction in weight was somewhat less—about 15 per cent In both cases the retardation of growth persisted after birth. Later experiments demonstrated that while plumbism in the male does not increase the number of stillbirths, intoxication of the female by lead does cause an increase which is out of all proportion to the severity of the poisoning Cole and Bachhuber (84) have reported similar observations on rabbits and fowl. Paul's (355) investigations seem to indicate that lead injures only the germ cells formed during intoxication, for he reports that pregnancies were normal after exposure had ceased

It is, therefore, clear that lead does not kill the germ cells, but only harms them so that the fetus either dies or is undersized. Injury is possibly caused in part by a reaction similar to that which occurs in red blood cells and in muscles But that it may also result from a special susceptibility of the fetal tissues to the toxic action of lead has been suggested by the work of several investigators (361) (260) (293) (152) who report that lead is present in the tissues of the fetus when the mother is "leaded" The recent work of Blair Bell (32) is partly based upon such observations. In our few experiments, however, no lead was found in the young of "leaded" animals (table 36).

In explaining the premature deliveries and stillbirths which occur so characteristically during plumbism, still another mechanism is involved. Lead reacts with the smooth muscle of the uterus much as it does with the smooth muscle of the intestines during lead colic, and in this way probably causes abortion The action of diachylon ointment is doubtless of this nature (see also page 124)

XIII. THE LEAD LINE

The most constant sign of plumbism and one which appears in no other condition, except possibly bismuth poisoning (237), is the so-called Burtonian or lead line in the gums This has certain quite definite characteristics: It occurs typically in the gingiva near the border of the teeth, and is seen usually near decaying teeth, either in the gum or in the mucosa of the lip or cheek opposite these. More-

over, rapid caries seems to develop whenever a lead line appears near teeth apparently in good condition That the pigmentation is not on the teeth themselves, may be easily demonstrated by inserting

Fig 29 Microscopic Drawing Demonstrating [illegible] of Lead Line

paper between the teeth and the gum. Furthermore as the true lead line can never be rubbed off it must be within the tissues and not be merely a surface deposit of pigment.

Microscopic examination shows that the line is composed of black, irregular amorphous granules which are entirely subepithelial They occur in the interior and the walls of blood vessels, in connective tissue fixed cells, in macrophages, and especially in the papillae of connective tissue which run to the base of the epithelium (124) In figure 29 is shown the lead line as it typically appears under the microscope

To explain the formation of this sign which is so indicative of lead absorption and so important in the diagnosis of lead poisoning, several theories have been advanced All of these assume that the coloration in the tissues is caused by the interaction of dissolved lead and hydrogen sulphide which results in the formation of black lead sulphide How the hydrogen sulphide is formed, how lead is brought to this region, how lead and hydrogen sulphide come into contact with each other, and by what chemical processes the lead sulphide is formed, are questions which must be answered before any explanation of the lead line can be complete

Hydrogen sulphide is a product of putrefaction, especially of animal proteins Thus there are two sources of this gas in the mouth the debris of protein food caught between the teeth, and the decaying gums and teeth themselves This explains one of the reasons for the clinical observation that a lead line rarely forms if teeth are cleaned regularly. In this connection it may be of interest to state that, even when severely poisoned rabbits, herbivorous animals, never have this lead line, while in cats, carnivora, it almost invariably appears very soon after administration of lead

The mechanism by which lead is transported to the gums is less easily explained

That lead may be directly absorbed by the mucosa of the mouth has been demonstrated by Blumgart (40) Constantin Paul (355) was able to produce a lead line even by the local application of lead to the gums To answer Drisolle's (101) question whether the lead line may be a manifestation of a local action rather than of general absorption of lead, Paul painted the gums of four women with a solution of lead acetate Their teeth were in perfect condition, and all other exposure to lead was prevented After the first application of the solution, the teeth darkened and an incipient lead line could be seen, after the fourth this had become quite definite It persisted for months, although there were no other signs of lead poisoning This experiment could not be repeated on animals.

The suggestion has been made that lead is excreted by the salivary glands and accumulates in the mouth (365) (383) (400) If this actually occurs, lead could easily be reabsorbed by the mucosa Schmidt (400), indeed, believed that the localization of the lead line is due to the special affinity of certain areas of the gum for lead excreted from the salivary glands Although small quantities of lead have been found present in the salivary glands both by Pouchet (365) and in this laboratory in one cat, and a metallic taste is often complained of by individuals suffering from lead poisoning, evidence of such excretion has never been obtained Moreover, the pigmentation frequently occurs in teeth which are not near the salivary ducts Although lead may reach the gums directly under some conditions of exposure, this is probably neither the necessary nor usual process by which lead is localized in the gums The appearance of a lead line in our cats after subcutaneous administration of lead is evidence that the lead may reach the gums either by excretion from the salivary glands or by the circulation But the microscopic examination which shows that the black granules never appear scattered throughout the mucous membrane, as they would if lead were absorbed through the mucosa, but are always subepithelial in position, is a definite indication that lead is brought to the gums by the circulation

Quite a different theory was advanced by Meillère (293) who believed that phagocytes carry lead to the gums for elimination This view was based on the fact that many leucocytes containing particles of lead sulphide may be seen within the tissues of the gums But the occurrence of such phagocytes probably indicates not that lead is transported to the gum, but rather that it is being removed from this region In fact, similar phagocytosis occurs when insoluble particles of lead are removed from subcutaneous and pulmonary tissue or when mixed *in vitro* with leucocytes This has been demonstrated by Carles (67) and by our experiments in which lead was administered by lung, and by the work of Fine (137)

In all probability lead, as the tertiary phosphate in colloidal solution, is carried to the gums, just as it is to all other parts of the body, by the blood And wherever it meets the hydrogen sulphide which is formed in the mouth and diffuses through the mucosa a precipitate of lead sulphide is formed The obvious objection to this idea is that

lead sulphide could hardly form if lead occurs in the blood as lead phosphate, a more insoluble salt However, as H_2S accumulates from decaying material in the mouth, more lead must change from phosphate to sulphide in accordance with the law of mass action. This would be greatly accelerated if the reaction of the tissues was more acid than that of the blood for in an even slightly acid medium, tertiary lead phosphate is gradually converted to the di-lead salt which is about fifteen times more soluble than the sulphide

There is evidence that changes occur in the gum before the lead line is grossly visible In human beings (440) and also in cats, distinct localized hyperemia has been seen to precede the lead line In this condition the gums are sore and bleed easily Stephens believes that irritation causes enlargement of the papillae at the edge of the gum by engorgement of the vessels and that lead "finds its way" to this hyperemic region and "leaves its mark" on the gums However, it seems more probable that the redness is merely an inflammatory reaction to the foreign particles of lead sulphide precipitated in the tissue. Microscopic examination verifies this opinion. In one of our cats the vessels in the inflamed gums appeared distended, and many red blood cells and polymorphonuclear leucocytes filled the submucosa, giving every evidence of marked acute inflammation. Scattered throughout this inflamed area black granules, presumably of lead sulphide, were seen In another specimen from an animal with such irritated gums but with no visible lead line, the chemical test for lead was positive From these observations the conclusion may be drawn that the deposition of lead salts in the gums begins before the lead line is grossly visible, and that the discoloration is evident only after the deposit becomes sufficiently great

The possibility of an interrelation between the lead line and caries of the teeth is important practically As early as 1870, Lewy (264) found that lead is deposited directly in the teeth just as in the other bones, and he believed that this might be a direct cause of caries Recently lead has again been found in teeth as well as in the rest of the skeleton (page 75), but whether this is a factor in the development of caries or the lead line, is as yet unknown The notoriously poor teeth of lead workers seem to indicate that the deposition of lead in the teeth may be important in causing decay. It might

be thought, however, that caries in the teeth of workmen is due to their unhygienic habits rather than to the action of lead However, excellent evidence of the probable deleterious effect of lead on teeth is seen in cats who, under normal conditions, always have perfect teeth, but who develop rapid and marked caries when exposed to lead

XIV LEAD COLIC

Although not in itself of serious consequences, lead colic is the most painful and dreaded symptom of lead poisoning and presents a difficult diagnostic problem The physiological mechanism and more particularly the direct cause of colic are as yet very little understood because of the scarcity of experimental data

There is, however, general agreement that any condition of colic is due to marked constriction of the small intestine This fact was first emphasized by Mackenzie (273), and post mortem examinations of animals dying from acute lead poisoning show that intense contraction of the gut actually occurs (337) The various explanations of the exact mechanism by which lead causes this spasm of the intestine fall into two general groups (*a*) Lead may act on one of the nervous structures supplying the intestine, or (*b*) may directly affect the smooth muscle itself A detailed presentation of each of these theories is hardly possible, for it involves a careful examination of the exact methods used in each case and would be essentially profitless This is especially true of the ideas concerning the nervous origin of colic Every possible nerve center and ganglion has been suggested as the site of lesion, usually with very little evidence Among the structures mentioned are the sympathetic nerves (386) (217) (293), the coeliac ganglion (319), the vagus center (282), the submucous and myenteric plexus (274), and the ganglia in the intestinal wall (199) In addition, the literature contains many suggestions which can only be characterized as flights of fancy, such as reflex constriction due to myalgia of abdominal muscles (268) and irritation by hardened feces (464)

Before analyzing the various theories which have been advanced to explain lead colic, a consideration of the physiology of colic, of any origin, is desirable If due to muscular constriction, colic must

result from either. (*a*) Stimulation of the vagus, through its center in the medulla, the nerve itself, or its endings, (*b*) inhibition of some part of the sympathetic nervous system, or (*c*) stimulation of muscle directly. A physiological observation, made by Cannon (64), should be mentioned here. He produced paralysis of the esophagus by sectioning the vagus and observed marked prolonged contraction of the cardiac sphincter of the stomach He states that since this spasm occurs after vagotomy, it is independent of extrinsic innervation The bearing of this phenomenon upon an explanation of lead colic depends upon the relation between loss of intestinal motility and spasm (page 165).

At this point mention should be made of a few of the more striking difficulties encountered in investigating lead colic before presenting the experimental and clinical evidence which has proved of value The most baffling of these is to obtain from animals satisfactory data on such a subjective symptom as the pain of colic Another and less well appreciated complication is the difference between experimental and actual conditions of exposure Attempts which are frequently made to obtain strikingly positive results by intravenous administration of extremely large doses of lead salts, or by exposure to such very toxic organic compounds as lead tri-ethyl acetate, can, of course, hardly provide data from which to draw legitimate deductions as to conditions in human plumbism From pathological studies of lead colic, just as of other manifestations of plumbism, unsatisfactory conclusions have been drawn on the basis of fragmentary and uncontrolled data

With these facts in mind, some of the more important contributions to this question may be considered in detail

Wassermann (481) poisoned cats with lead and examined the gastro-intestinal tract by x-ray He found that the hyper-motility of the small intestine, with a disappearance of the usual beaded picture which first occurred, was followed by a broadening of the contents, which suggested loss of tone In the large intestine the contraction rings and peristalsis were marked and this was accompanied by a delay in the excretion of the contents Wassermann thought that colic is apparently associated with spasm of the small rather than of the large intestine and that during attacks the small intestine is contracted to the size of a thread

X-ray examinations of one of our patients during a rather severe attack of colic gave results which seem to correspond to Wassermann's description Definite areas of intestinal spasm were seen and the stomach which appeared hypertonic, exhibited exaggerated peristalsis Six hours after the barium meal, the head of the barium column was held in the ileum, in twenty-four hours all the barium had accumulated in the cecum and ascending colon which appeared tremendously dilated, and none had passed the hepatic flexure Over this area there was some tenderness An enema given five days later showed no obstruction at the hepatic flexure, and another x-ray examination a week later showed nothing abnormal

Although Wassermann made no microscopic examinations in his experiments, he attributes colic to sclerosis of the sympathetic ganglia, which has been reported by other investigators (see page 115) He believes this would permit unchecked vagus activity to cause intestinal spasm To this view there are two objections First, there is not sufficient evidence that lead causes a lesion in the sympathetic ganglia, and second, such a lesion, if it did occur, would impair the function of the ganglion permanently, whereas lead colic is a temporary condition from which recovery may be complete

Somewhat similar conclusions were reached by Hirschfelder and his co-workers from experiments on intestinal peristalsis They inserted a petri dish into the abdominal wall of rabbits as a window through which the intestine could be observed, and injected lead acetate (5 mgm per kilo) into the circulation This was followed immediately by the development of intense intestinal peristalsis which was unaffected by section of the spinal cord or vagus nerve but was abolished by the injection or application of nicotine or atropine From these results they concluded that the spastic action of lead is due to its effect on preganglionic nerve fibers and not on muscle fibers They also investigated the effects of nitrites on this tonic spasm and thought that they act directly on the smooth muscle of the intestine and do not relieve colic merely by general vaso dilation Several criticisms of this work should be made Such immediate and acute intoxication is hardly comparable to that which occurs in life Furthermore, such drugs as atropine and nicotine act on different structures and produce varying effects according to the strength of the dose Atropine

may affect nerve plexuses or smooth muscle as well as act on the vagus, nicotine in small doses may stimulate the sympathetic instead of depressing the ganglia (86). Vagus section and cord excision, of course, have no effect on the activity of muscle fibers themselves or on myenteric reflexes

Hanzlik (196) tried an ingenious experiment on the intact duck By inserting a small rubber balloon into the crop he was able to record peristaltic contractions on a kymograph They were more vigorous, rapid, and regular in lead poisoned ducks, and neither atropin nor epinethrin affected them, while chelidonin, which depresses smooth muscle independently of nerve endings, promptly inhibited the contractions He, therefore, concludes that the augmentation of contraction due to lead was caused by direct stimulation of the smooth muscle

In 1872 Kussmaul and Maier (243) suggested that an inflammation and ulceration of the gastro-intestinal tract caused by lead results in colic This view, when first presented, was based on a single post mortem examination, but ten years later Maier (274) supported the idea by experiments with rabbits and guinea pigs After the daily administration of lead salts in doses of 0 2 gram, degeneration of gland cells, hemorrhages, venous stasis, and sclerotic degeneration of the submucous and myenteric ganglion cells could always be seen in the intestine. While the afferent and efferent nerves appeared normal, the more central ganglia, such as the coeliac, often showed lesions. The severity of these changes suggests that the lead salt exercised a marked local action on the gastro-intestinal tract

From experiments with isolated smooth muscle from the gastro-intestinal tract, the uterus, the blood vessels and ureters, Siccardi and Dozzi (431) were led to believe that lead acts to a great extent on the muscle fibers and not on the nerves alone

These authors found that high concentrations of lead acetate increase the tone of isolated muscle strips but diminish the contraction waves Upon removal of the lead, muscular activity returns to normal Further support of the observations has also been given by Siccardi's (429) experiments with atropine, nitroglycerine, and nicotine From his results Siccardi concluded that colic may be due either to the hypertonicity of intestinal muscles caused by the action of small doses of lead, or to reflex constriction following

the hypotonicity of muscle and cessation of rhythmicity which larger doses occasion Unfortunately these experiments *in vitro* have little bearing on the action of lead within the body during chronic lead poisoning

But that lead may exercise effects directly on smooth muscle has been shown by unpublished experiments with Miss Dorothea E Smith (20) These were designed to determine whether lead acts on the myenteric nervous system or directly on the smooth muscle fibers themselves—a point not settled by Siccardi

Strips of circular muscle containing no ganglion cells were prepared from the small intestine by the method of Gunn and Underhill (181) and the effects of lead salts on these were studied by the method of Magnus, as used by Cannon and de la Paz (64) The temperature of the apparatus was maintained at 37°C and oxygen was continuously bubbled through the bathing Ringer solution After normal contractions were recorded from all the strips, lead-Ringer mixtures were added to some of them The rest were used as control preparations in the experiment Immediately after the experiment a microscopic search for ganglion cells was made Of twenty strips used in successful experiments, twelve were found to contain no demonstrable nerve cells, and on the five most satisfactory of these, lead was found to have the same effect that it does on longitudinal strips of smooth muscle and on strips containing nerve cells Therefore, the assumption that lead acts *in vitro*, in large part at least, directly on the muscle fibers and not on the peripheral ganglia appears to be justified

The effects of lead on smooth muscle as noted by Siccardi and confirmed in this laboratory by more than 110 observations are (*a*) inhibition of spontaneous contractions, and (*b*) an increase in tonicity. That these two phenomena are independent of each other is indicated in these experiments by the fact that in some cases small rapid contractions continue while the tonus rises markedly, while in others the rhythmical contractions cease although the tonus does not vary Changes caused by 1 mgm of lead acetate per 100 cc of Ringer solution, or 1 part per 100,000, are not always permanent, but may persist for a short time and be followed by gradual recovery of the muscle, even when the lead is not removed In tests of the relative toxicity of lead acetate, lead chloride and lead nitrate, no distinct differences between these salts were observed The addition of serum albumin did not markedly alter the results, but changes in the

hydrogen ion concentration caused striking variations At pH 6.5, as would be expected, lead exercised much less effect than at pH 4 8.

The action of lead on smooth muscle as described in these experiments is made very significant by the clinical observation that lead colic is almost invariably preceded and accompanied by obstinate constipation Tanquerel des Planches (453) lays great stress on this feature of plumbism in the twelve hundred cases which he reported Also Heubel (213) and Meillère (293) were both convinced of the distinct connection between colic and constipation, and Oliver (337) and Legge and Goadby (252) were impressed with the relationship Furthermore, none of our patients developed severe colic even when excreting relatively large amounts of lead. This was probably due to the fact that constipation is prevented as part of a hospital regime

Thus, there are two outstanding features of the mechanism involved in lead colic (*a*) Lead inhibits the motility of smooth muscle while it increases the tone, and (*b*) clinically colic is preceded by obstinate constipation As yet, sufficient data for a definite physiological explanation of lead colic have not been collected, but it is quite apparent that lead does not exercise a selective action on any of the nervous structures of the gastro-intestinal tract From a survey of the meager experimental data and the rather definite clinical observations, the most reasonable suggestion to explain lead colic is that lead acts directly on the smooth muscle of the intestine, producing increased tone and loss of motility, and therefore constipation results The griping pain of colic may be due to a reflex constriction which approaches such an amotile, hypertonic area, a condition similar to that found in intestinal obstruction (63b)

XV NERVOUS REACTIONS

Lead palsy. Paralysis is the most incapacitating manifestation of lead poisoning and is especially distressing because of its chronicity and because the muscles affected are usually those most used in routine work. As almost all investigations of this condition have been pathological and clinical in nature and have consisted of careful general and microscopic examination of the neuro-muscular apparatus, much detailed information about the late gross and minute lesions of palsy

has been collected From this, attempts have been made to deduce the process by which lead causes paralysis In order to evaluate these studies, it may be well to give an orderly and systematic summary of the various theories which have been offered and to mention some of their proponents For convenience in discussion, the pathological, clinical and physiological aspects of lead palsy will be considered separately

Pathology From a study of the morbid anatomy and histology of patients with lead palsy, and from the observations on experimental animals, theories of the mechanism of lead paralysis have been evolved which locate the primary lesions in every part of the neuro-muscular system

As long ago as 1879 Friedlander (149) considered muscular changes of first importance and described a degenerative myositis in which the sarcolemma was primarily involved and which was characterized by marked fibrous tissue replacement He believed that the involvement of nerves supplying the atrophied and degenerated muscles were secondary to a primary muscle injury Kast (231) was the only other investigator to commit himself definitely in favor of this view until Messing (301) pointed out quite clearly that careful microscopic examination always demonstrated that the lesions in the muscle are more marked than in any other part of the neuro-muscular apparatus Since the lesion is not a simple atrophy but really a specific inflammatory process, he concluded that lead must act directly on muscle and not merely interrupt trophic fibers by causing lesions in peripheral nerves

Other investigators have suggested that muscle plays only a secondary rôle in the etiology of lead palsy, and that lead acts primarily on the vascular system (218) (364) (381) (252)

According to them, lead directly injures the vessel walls so that they rupture with relative ease during muscular activity in both nerve and muscle Messing also believed that the essential lesion in muscle was vascular, but in his careful examination he found that the blood vessels were affected only in the muscles involved—a fact which throws doubt on the general vascular action of lead, and supports the view that lead acts directly upon muscle

Most pathologists, however, consider that the primary lesion of lead paralysis is in the nervous system The idea that it is in the

spinal cord has many supporters (120) (380) (91) (313) (335) (499) (360) (443) (479) (381) (341), most of whom base their opinion on the appearance of distinct degenerative lesions in the anterior horn cells, while another large group of investigators consider changes in the peripheral nerves to be of fundamental importance (245) (490) (342) (92) (172) (77) (265), and describe a distinct Wallerian degeneration in the nerves running to the paralyzed muscles. Déjérine-Klumpke (92) emphasizes the fact, which Gombault (172) first pointed out, that the lesion is distinctly peri-axial.

The conception, however, that the lesion of lead paralysis is essentially complex has been most generally accepted, even by those who believe that lead acts directly upon a particular part of the neurone (36) (406) (371) (478) (357) (138) (435) (173) (494) (89) According to this view, lead affects that part of the neurone which for the time being is rendered most susceptible by its functional condition, i.e, the *locus minoris resistentiae* (379) (173). Recently Hyslop and Kraus (224) have summarized the pathology of lead palsy and have concluded that the lesion is probably a neuronitis which may be manifested in any part of the neuro-muscular apparatus. In evaluating this pathological work, however, it is important to realize that all examinations were made after death, and in many cases only after palsy had persisted for several years In most of these investigations the attention of the pathologist was so focused on some special part of the neuro-muscular system that the rest was neglected, and usually it was the muscle which was ignored At best, post mortem examinations can give but a slight suggestion of the conditions which require dynamic methods of investigation. A satisfactory explanation, therefore, cannot be derived from pathological examinations alone.

Clinical observations. Quite early, investigators attempted to determine the pathogenesis of palsy by careful clinical examinations One of the first observations, that the extensors of the forearm are generally involved in peripheral palsy, suggested the idea that lead may be absorbed through the skin and thus injure the muscle by direct contact (280) (108) (276) (392) As soon as knowledge of the absorption of lead accumulated, however, this idea became untenable Déjérine (91), Remak (380), and von Monakow (313) believed that

only central lesions could cause paralysis of functionally correlated muscles and explain the symmetrical character of the lesions But more recent observations have shown that the peculiar localization of palsy has an altogether different explanation and that the symmetry of the lesions is over emphasized There is some clinical evidence which points toward the muscle itself as the site of the action of lead A reaction of degeneration is obtained from direct stimulation of paralyzed muscles as well as of nerve, but that this may merely indicate a secondary muscular atrophy following a nerve lesion must be kept in mind The susceptibility to fatigue and the general weakness of patients suffering from plumbism have long been recognized by clinicians The occurrence of paralysis in fatigued muscles is said to have been first pointed out by Meyer (304) in 1854, and definitely described by Moebius (312) in 1886 Through the work of Edinger (105) this observation has been made the most important single clinical contribution to the explanation of palsy and has been accepted by almost every student of plumbism (484) (455) 337) (168) In an attempt to explain this observation, Hitzig) (218) in 1868 suggested that since the region with the greatest blood supply must receive the most lead, the fatigued muscles in which the circulation is greatly increased are necessarily exposed to the largest quantity of lead Such an explanation, however, gives no clue to the actual mechanism by which lead acts on muscle tissue It is evident, therefore, that this interrelationship of fatigue and the development of lead palsy can be solved only by chemical and physiological investigation

Physiology The importance of the action of lead on the physiology of the neuro muscular system was apparent to some of the earlier investigators, and in fact as long ago as 1877 Muson (325) is reported to have demonstrated palsy in the hind limbs of frogs after immersing the animals in water containing lead He found no lesion in either nerve or muscle Since that time some interesting and suggestive experiments have been performed which constitute the basis of conflicting theories These are fully discussed in an article by Reznikoff and Aub (385) Harnack's results (199) which were partly due to the ethyl group of the organic compound he used, led him to believe that lead palsy is caused by direct injury to the muscles, and Cash's un-

controlled experiments (71) confirmed his observation that after exposure to lead the relaxation time of muscle is prolonged. Mellon (296) also obtained data which seem to support the theory that lead produces a definite functional lesion in muscle Dozzi (100) on the other hand, suggested that lead might affect isolated nerve as well as muscle and obtained results which apparently strengthened his theory, but too much reliance must not be placed upon these observations because he used very concentrated lead solutions of unknown acidity which contained no other salts. This brief summary indicates that our knowledge of the physiological action of lead on the neuro-muscular system is very slight Such ignorance could in the past be accounted for partly by lack of knowledge of the physiology and chemistry of muscle, which recent investigation has removed to a considerable extent (139) (140) (141) (142) (144) (219) (216) (305) (115) (116), and partly by a lack of accurate chemical and physiological knowledge of the action of lead within the body.

Some of our investigations have suggested a means of studying the physiological aspect of lead palsy In experiments dealing with the effect of lead on red blood cells (15) (16) (17), it was found that lead so alters the surface of the corpuscle that its permeability to water is changed. The question arises, therefore, as to whether lead may not act on the surface of muscle in a similar manner and thus change its permeability.

Of the various methods of studying the passage of substances through the surface of muscle (142) (219) (216) (305) that devised by Embden (116) is the most satisfactory for our purpose It is based upon his observation that an increase in the diffusion of inorganic phosphate from muscle is due to an increase in the permeability of the cell membrane Because of the ease of determining the quantity of inorganic phosphate in solution we have employed this method in a study of the effect of lead on the permeability of the surviving muscle of the frog (385).

In our experiments, isolated muscles from frogs were placed in beakers containing Ringer solution The quantity of inorganic phosphate which diffused into the bathing fluid was determined hourly until a low and practically constant value was obtained One of the muscles was then placed in Ringer solution containing lead (0 05 mgm Pb per cubic centimeter) and

allowed to stand for one hour, while the other was kept as a control in regular Ringer solution Both muscles were then transferred hourly to fresh Ringer solution and the inorganic phosphate content of corresponding solutions from the control and "leaded" muscles was determined The analysis for inorganic phosphate was carried out by a modification of the Bell-Doisy blood method in which the protein precipitation stage was omitted, and the entire quantity of fluid was compared with standards prepared simultaneously with the unknown Nessler tubes were substituted for a colorimeter, in order to decrease the error in matching the very faint colors produced by the small quantities of phosphate From these results, a curve of phosphate diffusion was obtained for each muscle and any difference caused by the action of lead could be determined

Thirty-four out of thirty-five experiments showed quite definitely that the diffusion of inorganic phosphates (expressed as milligrams of P per gram of muscle per hour) increased markedly after "leading" Control muscles underwent no such change, except for a slight increase following stimulation in some experiments Their diffusion followed the same course as that in Fletcher's CO_2 experiments, which was characterized by an immediate rapid decline succeeded by a very gradual decrease in the rate of diffusion A summary of the results of all the positive experiments shows that after exposure to lead the average rate of diffusion rose 320 per cent above the lowest value In only two cases was the increase less than 100 per cent while in nine it was between 100 and 200 per cent, in ten, between 200 and 300 per cent, and in two experiments more than 1000 per cent Figure 30 illustrates one of the most striking experiments and figure 31 is a composite curve of all the 34 positive results After the muscles were removed from the solution of lead, there was considerable variation in the length of time which elapsed before the diffusion of phosphate became maximal, but as a rule this was about three hours The duration of maximal diffusion was usually only one to two hours, but in most cases the rate did not return to the low original value during the experiment

Other factors than exposure to lead such as stimulation, injury, or rigor may alter the rate of phosphate diffusion Control experiments showed that injury was a negligible factor in this work and that stimulation produced only a very slight rise in some cases Rigor was avoided by providing a constant supply of oxygen and by

preventing undue fatigue Although the relationship between rigor and the muscular changes caused by lead cannot be considered in detail here, it will be of interest to remember that rigor is supposed to be due to the acid products of fatigue Examination of muscles after exposure to lead demonstrates that they are shrunken, of rubbery consistency, and lusterless—in fact, quite comparable in appearance to muscles in rigor.

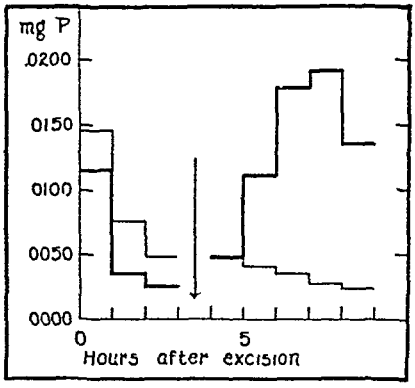

FIG 30

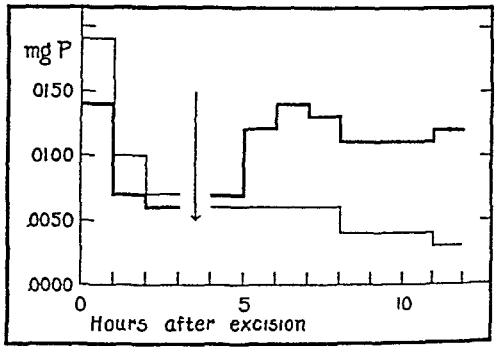

FIG 31

FIG 30 CHART SHOWING EFFECT OF LEAD UPON THE RATE OF DIFFUSION OF INORGANIC PHOSPHATES FROM MUSCLE

The heavy line represents the rate from "leaded" muscle, the light line that from the control muscle The arrow represents period of exposure to lead (0 05 mgm Pb as $PbCl_2$ per cubic centimeter Ringer solution)

FIG 31 COMPOSITE CHART SHOWING EFFECT OF LEAD UPON THE RATE OF DIFFUSION OF INORGANIC PHOSPHATES FROM MUSCLE

The heavy line represents the rate from the muscle exposed to lead solution, the light line that from the control The arrow represents period of exposure to lead (0 05 mgm Pb as $PbCl_2$ per cubic centimeter Ringer solution)

Some other observations merit consideration If a "leaded" muscle is treated with ammonium sulphide and examined under the microscope, all the lead appears on the surface and none has penetrated into the muscle Another fact is also of interest in this connection When a muscle is exposed to lead there is a change in the reaction of the surrounding solution, in one case from pH 6 5 to 4 8, in a second experiment to pH 5 5, and in a third to pH 6 1 Such an increase in acidity agrees with the results of our work on the effect

of lead on red blood cells, which show that soluble lead salts unite with the inorganic phosphate to form insoluble lead phosphate with the liberation of free acid To determine the effect of acid on the permeability to inorganic phosphate, acid Ringer solution was added to muscles In one case, in which the pH of the Ringer solution was 4 5, the permeability of the treated muscle remained practically like that of the control, but in two experiments in which the pH of the Ringer solution was 3 5, the rate of phosphate diffusion increased markedly at once The maximum increase was very brief, but throughout the experiment the rate of diffusion remained constant and did not decrease as did that of the control In one case it remained twice as high as the original rate before exposure to acid The maximum increase in diffusion due to acid was 140 per cent in one of these experiments and in the other 520 per cent That this type of experiment is not comparable to those in which the muscle is exposed to a lead salt, must be remembered, for in the latter case acid is produced in the tissue of the muscle and the buffer phosphates must be diminished by their interaction with lead But these acid experiments offer further evidence that when lead salts act on isolated muscle the reaction which probably occurs is similar to that with red blood cells, and as a result there is a marked change in the permeability to inorganic phosphate That this is due to an alteration of the surface and not to the break-down of increased amounts of organic phosphate within the muscle, is indicated by the increased rate of diffusion after removal of the muscle from the lead solution and by the persistence of this increase for several hours, usually throughout the experiment

Such a striking change in the permeability of the surface of muscle to inorganic phosphate suggests that lead may have a demonstrable effect on the activity of isolated muscles To determine this, experiments were performed on nerve-muscle preparations (sciatic-gastrocnemius) in specially constructed chambers which are fully described in a detailed account of the experiments (385) These permitted simultaneous stimulation of either both nerves or both muscles since the electrodes were arranged in series, and also allowed the Ringer solution to be drawn off for analysis of inorganic phosphate

Thirteen experiments were performed with tetanic stimulation In five of these no difference could be seen in the rate of onset of fatigue

in the control and "leaded" muscles, probably because the great amount of work done fatigued both muscles very rapidly and caused rigor In no case, however, did the control fatigue more rapidly than the "leaded" muscle In eight experiments in which the control muscle remained in good condition, the "leaded" muscle fatigued very

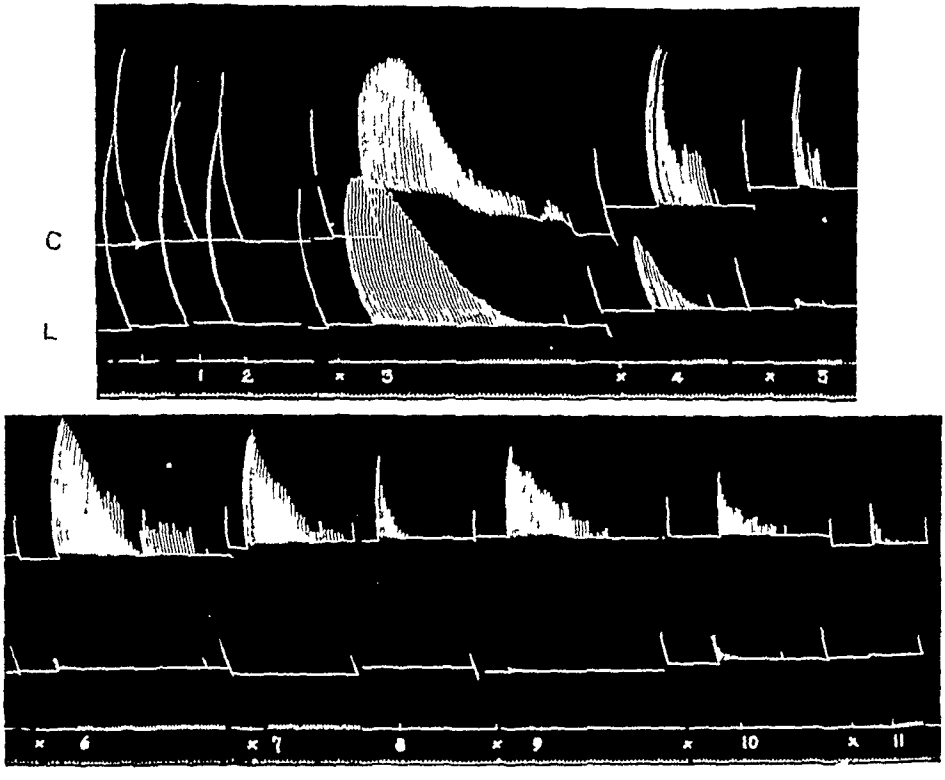

FIG 32 GRAPHIC RECORD OF MUSCULAR RESPONSE TO INTERMITTENT TETANIC STIMULATION BEFORE AND AFTER "LEADING"

C is the tracing made by the control muscle, *L* is the tracing made by the "leaded" muscle (0 05 mgm Pb as $PbCl_2$ per cubic centimeter Ringer solution) The figures indicate the periods of stimulation, the crosses represent readjustment of the after loading of the muscles

rapidly and usually did not recover Figure 32 illustrates one of these in which the muscle was stimulated directly and the rate of phosphate diffusion was determined hourly (fig 33). This experiment demonstrated several interesting facts The "leaded" muscle fatigued much more rapidly than the control and failed to recover The excellent initial contractions obtained after removal from the

lead-Ringer solution demonstrate that the action of lead is manifested only after muscular activity Comparison of the diffusion curve and muscle tracing shows that the greatest quantity of phosphate diffuses when the muscle is most fatigued during the first, second, and especially the third hour after exposure to lead

Because tetanic stimulation seemed to fatigue control muscles so readily, a series of experiments (ten) was performed with make and break stimulation These tests showed very distinctly that after an

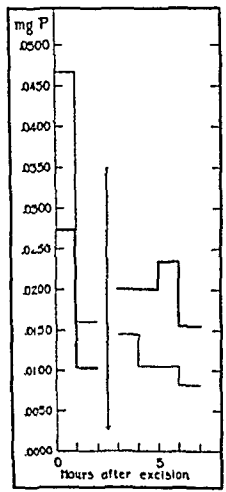

FIG 33 CHART SHOWING VARIATIONS OF THE RATE OF INORGANIC PHOSPHATE DIFFUSION FROM NORMAL AND "LEADED" MUSCLES DURING TETANIC STIMULATION

The heavy line represents the rate from the "leaded" muscle (0 05 mgm Pb as $PbCl_2$ per cubic centimeter Ringer solution) the light line that from the control muscle

initial period of sustained uniform contraction, the "leaded" muscle fatigues more rapidly and completely, and recovers with much greater difficulty than the control in every case (fig 34) From these investigations the conclusion may be drawn, therefore, that lead interferes with the function of isolated muscle very markedly Stimulation produces fatigue much more rapidly after exposure to lead, while recovery, difficult at best, in most cases does not occur at all

The next question to be considered is the effect of lead on nerve Because of the clinical designation of lead palsy as a peripheral neuritis as well as the occurrence of lesions in peripheral nerves, the impression is prevalent that lead acts specifically on peripheral nerve The

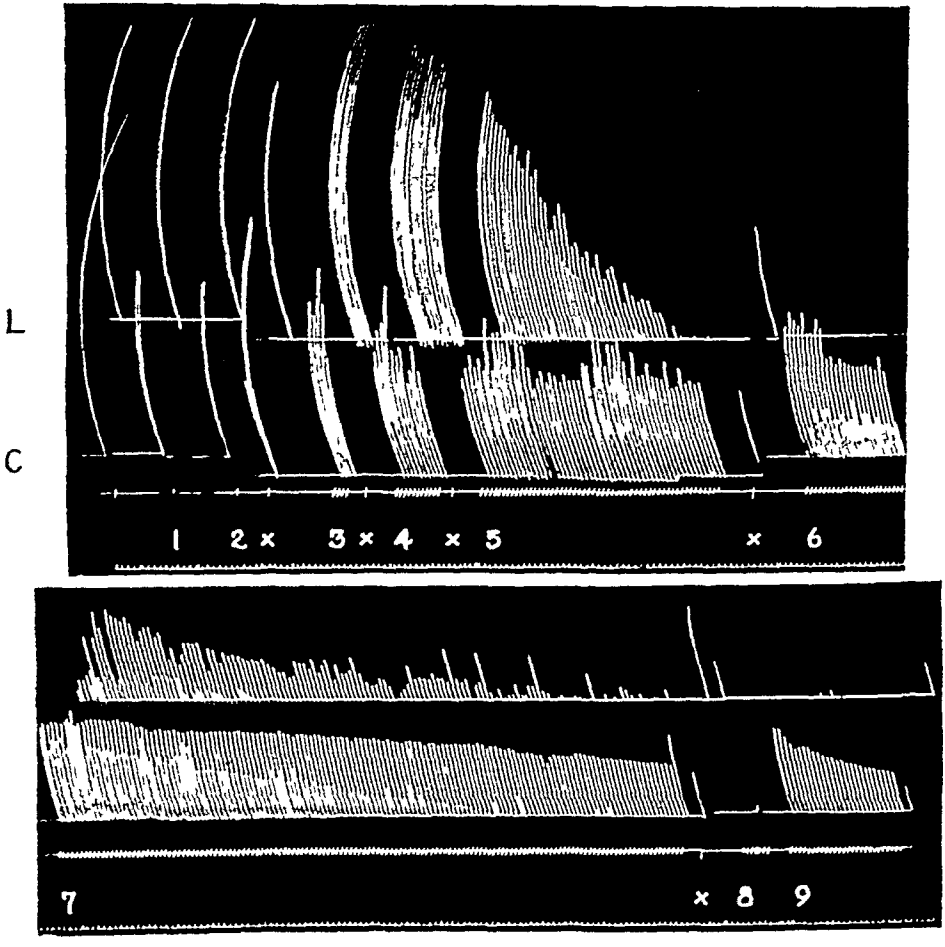

FIG 34 GRAPHIC RECORD OF MUSCULAR RESPONSE TO MAKE AND BREAK STIMULATION

L is the tracing made by the "leaded" muscle (0 05 mgm Pb as $PbCl_2$ per cubic centimeter Ringer solution), *C* that of the control

only experimental study of this problem reported in the literature was made by Dozzi (100) which has already been criticized

The action of lead on isolated nerve has been studied in this laboratory (385) As judged by muscular response, the conductivity

of the "leaded" nerve was exactly the same as that of the control even after exposure to concentrations of lead as high as 0 46 mgm per cubic centimeter—the largest quantity soluble in Ringer solution of pH 6 5 Therefore, as far as such experiments can determine, lead produces no decrement in nerve conduction To check these results, an attempt was made to determine by the string galvanometer the action currents of both nerve and muscle as a delicate index of function In one of a pair of nerve-muscle preparations the nerve was exposed to lead, in the other the muscle One experiment demonstrated quite distinctly that after fatigue the action current of the "leaded" muscle diminished rapidly and soon disappeared entirely, while that of the control muscle returned to normal within a short while and persisted until the end of the experiment Similar undiminished action currents were obtained from both normal and "leaded" nerves Thus, the distinct inhibitory action of lead on isolated muscle and the lack of deleterious action on nerve is confirmed

In the explanation of lead palsy, as it occurs in life, the experiments described serve merely as preliminary and suggestive observations which must be correlated with the production of palsy in living animals if they are to have any important practical significance The ample evidence that in life lead paralysis develops in muscles which are fatigued (304) (312) (105) (484) (455) (337) links the problem of lead palsy with the chemistry of muscular fatigue and suggests a method of investigation in intact animals Consequently, a series of experiments was carried out in an attempt to obtain explanatory data In order to produce unilateral fatigue, weights were attached to the dorsal surface of the right forepaw of severely "leaded" and control cats, and the animals were exercised daily in a revolving drum Marked fatigue was thus obtained in the extensor muscles of the right forepaw by constant raising of the weights When a distinct difference between the weighted limb and the other became apparent, the animal was anesthetized and the thresholds of these muscles and their nerves were determined The results of these mammalian experiments are very striking After the administration of lead all the cats lost weight very rapidly and within one or two weeks showed distinct lead lines and became fatigued by exercise very easily The weighted foot showed distinct signs of weakness after about two weeks

of exercise This was manifested by difficulty in extending the foot when the weight had been removed for twenty-four hours, and was especially marked if the animal was held by its neck and permitted

FIG 35 PICTURE OF CAT SHOWING PALSY OF RIGHT FOREPAW

to reach for the top of a window ledge Figure 35 illustrates the flexed position of the right forepaw in one of the "leaded" cats Some of the animals limped decidedly in walking When the weights were still attached, the contrast between the "leaded" and control animals

was even more striking Those suffering from plumbism could not lift the weighted paw without great effort and then only with the aid of the upper leg muscles, while the control animals lifted both paws with equal ease After running in the drum, these differences were accentuated The "leaded" cats tired much more quickly, and to a greater degree than did the normal animals, and exhibited a very striking fatigue of the weighted limb, which usually completely prevented extension of the paw They took shorter steps with the weighted limb while running, and seemed to lean toward the left This picture fits almost all of our "leaded" cats in practically every respect

One of the most striking features of these experiments is the similarity between the threshold of the unweighted muscles of the "leaded" animals and that of both unweighted and weighted muscles of the controls when stimulated by nerve The threshold of the weighted muscle of the "leaded" animals to stimulation through nerve was always very much higher whenever the muscle was distinctly weaker than that of the opposite side (four cases) The relative difference between the two sides was also apparent with direct muscular stimulation in most of the cases tested

Proper appreciation of these experiments requires a brief consideration of the chemical relationship between lead in the body and the products of muscular activity Lead is deposited in the bones as insoluble lead phosphate (see page 43) but is mobilized by various changes in metabolism As is discussed elsewhere (section IV), lead probably acts in this way because the phosphate is very soluble in acids, especially in lactic acid which is liberated in large amounts during muscular activity, particularly during fatiguing exercise (387) (26) Therefore, when lead phosphate circulates through muscle undergoing vigorous exercise, it must be dissolved to a considerable extent by the lactic acid and thus be converted to lead lactate

If such a soluble lead salt as the lactate comes into contact with a cell, it can unite with the inorganic phosphate present at the surface (129) and form insoluble lead phosphate and free lactic acid In work already reported (17) it was suggested that the precipitation of this insoluble lead salt, which is accompanied by the liberation of free acid and the removal of buffer phosphate, changes the colloidal

state and the properties of the cell surface There is considerable evidence that this occurs in muscle Clinical observation has established the fact that the muscles paralyzed are those which are most used. As far as is known, only muscle and not nerve becomes fatigued; and experiments *in vitro* have demonstrated that lead does not interfere with the function of isolated nerve, whereas it does affect muscle markedly. This change in muscle function is preceded by an alteration of the surface permeability, and it is therefore very probable that lead acts upon muscle just as upon red blood cells, by changing the permeability of the surface

Thus these considerations afford a possible explanation of the development of lead palsy Lead is transported by the blood as an insoluble phosphate in colloidal state In regions of muscular activity this is dissolved by the excess lactic acid which diffuses from fatigued muscle cells and is converted into lead lactate As the soluble lactate comes into contact with inorganic phosphate at the surface of muscle cells, the lead is reprecipitated as insoluble phosphate—a reaction dependent upon the relative concentrations of lactate and phosphate. This work, therefore, suggests that the physiological lesion of lead palsy is in the muscle itself and that those muscles which are fatigued are most susceptible to lead paralysis. Furthermore, it suggests that the susceptibility to lead palsy depends on the chemical reactions between lead and the metabolic products formed during muscular activity.

The question of how to correlate these results with the pathological and clinical observations naturally arises There is no doubt that distinct lesions occur in the peripheral nerves and spinal cord in lead palsy. That a lesion in a neurone is followed by peripheral degeneration is well known and is the foundation for the opinion that palsy is essentially of central origin But the occurrence of distinct primary lesions other than atrophy in the muscles and simple Wallerian degeneration in the peripheral parts of the nerves, demands some explanation of primary peripheral injury and secondary central degeneration This question troubled even the early workers

Putnam (371) thought that central changes might be secondary to peripheral injury although he offered no explanation, and indeed this has

been suggested by all investigators who believe that lead paralysis is essentially a neuronitis According to Meillere (293), the most vulnerable part of the neurone is affected by lead, and a lesion once formed may spread either centrifugally or centripetally Although Remak (380) considered palsy to be of central origin, he stated that for the most part lesions are present in the muscles and in the peripheral parts of the nerves and that they become less marked centrally This was expressed even more positively by Legge and Goadby (252) who said that the more recent the palsy the more severe are the peripheral lesions, and that as the condition becomes more chronic the central lesions are more marked Although the exact mechanism by which peripheral injury causes central lesions is not known, suggestive work by Orr and Rows (345) possibly throws some light on the subject By experiments with tetanus toxin, bacteria, and dyes, they demonstrated a lymphogenous drainage from the periphery up the nerve to the cord, and the lesions which they described were in the adventitia around the veins and capillaries, and seemed very much like the lesions characteristic of the early stages of various forms of peripheral neuritis Walshe (480) explains the mechanism of diphtherial paralysis on the basis of these investigations This work, however, is merely suggestive, and further investigation is necessary before such an explanation can be applied to lead palsy

In conclusion it must be pointed out that it is impossible to explain all the phenomena associated with lead paralysis Although under ordinary conditions lead may have no specific predilection for any tissues except bone, it may produce a very marked effect upon certain tissues when their physiological conditions change Thus, the action of lead on quiescent and on fatigued muscle may be very different In the light of the more recent work on the chemistry of muscular activity and of lead within the body, it seems probable that the physiological lesion of lead palsy is in muscle The occurrence of pathological changes in nerve and spinal cord to which so much importance has been attributed by older workers, may, according to more recent views, simply result from peripheral injury Whatever remote and indirect lesions lead may produce, it is probable that in lead palsy there is a close chemical and physiological relationship between the action of lead and muscular activity, which may have great significance in many clinical and therapeutic problems

XVI MENINGO-ENCEPHALOPATHY

Perhaps the most dramatic manifestation of lead poisoning and at the same time one which is of the most serious prognostic import is the development of acute mental changes This condition, known as encephalopathy, was described as early as 1837 by Grisolle (178). Many theories, which point to various structures as the site of lesions, have been advanced to explain the etiology of the symptoms Encephalopathy has been ascribed to (*a*) cerebral arteritis (463), (*b*) cerebral arteriosclerosis (222), (*c*) cerebral anemia (390) (213) (293) (225) (300), (*d*) punctate hemorrhages due to arterial and venous degeneration (252), (*e*) uremic manifestations caused by the action of lead on the kidneys (88), (*f*) the direct action of lead on the cortical cells (see Pathology), and (*g*) to meningeal lesions (315) (316) (332) (50) (466) (203).

Analysis of these theories shows that some may be quite readily discarded. The isolated observations of arteritis and arteriosclerosis have never been substantiated The suggestion of other vascular changes as causative factors has more foundation Some evidence has been presented to support the idea that cerebral anemia, due to spasm of the vessels, is the basis of encephalopathy Siccardi and Dozzi (431) found that isolated arteries shrink in solutions of lead salts, but it is hardly probable that lead can thus affect the blood vessels *in vivo* (see Pathology). Although lead is generally considered a vasoconstrictor and some believe that clinical conditions may be explained by this action (206), it has never been definitely shown that lead causes a general rise of blood pressure (131). Mason (282) observed that after the injection of lead tri-ethyl acetate into animals the immediate fall of blood pressure was followed by a marked rise But it must be remembered that lead tri-ethyl acetate itself has a toxic action quite distinct from that of lead Furthermore, Page (350) believed that in workers who have been exposed to lead, hypertension usually is accompanied by some renal impairment The typical behavior of patients suffering from encephalopathy is another objection to the theory that cerebral anemia is responsible for the condition Since anemia always causes unconsciousness, it cannot be the basis of sustained excitability, which is so frequent during this manifestation

of plumbism as to be almost characteristic General secondary anemia, however, is a common sign of lead poisoning (see page 188) and might account for the punctate hemorrhages to which Legge and Goadby attribute lead encephalopathy Their hypothesis which is based on a few experimental observations and principally on a clinical report by Mott has already been discussed in the section on Pathology, and no further comment is required to indicate its inapplicability to the mental symptoms of plumbism

That encephalopathy may be of uremic origin, has never been substantiated by renal examination (see Pathology) The pathological picture, in spite of clinical opinion (493), blood pressure measurements, urine analyses, and blood chemistry determinations has rarely pointed to the kidney as the immediate etiological factor in encephalopathy (390) That lead acts directly on the cortical cells is suggested by the presence of lead, although in very small amounts, in the brain But the relatively slight damage seen in pathological examinations induced Meillère (293) to point out that "the violence of the symptoms observed contrasts singularly with the prompt re-establishment of apparent integrity " He believed that aside from some evidence of anemia in the gray matter no other microscopic or macroscopic lesions exist

The idea that lead encephalopathy may be principally a meningitis or meningopathy is of quite recent origin and is based on three separate pieces of evidence The pathological observations of Hassin (203), the experiments of Camus (61), and the changes of the cerebro spinal fluid reported by Mosny and Malloizel, Norton, Boveri, and Troisier

At post mortem examination, Hassin always observed a marked proliferative meningitis and in acute cases some round cell infiltration of the meninges The lesions in the brain, however, were relatively inconstant and slight, and consisted for the most part of vascular proliferation extending from the meninges In this connection it is interesting to note that almost all pathologists before Hassin had focused their attention upon the brain and had ignored the meninges

Camus is one of the few who have studied lead encephalopathy experimentally He injected lead into the subarachnoid space of a dog by cistern puncture and within two or three days symptoms of encephalopathy ensued—excitability, "hallucinations " and con-

vulsions just before death If he injected lead directly into the brain substance, no symptoms developed and only encysted necrosis of small areas of the brain resulted Neither did the mere administration of lead intravenously produce any mental symptoms, but of very great interest is the fact that simultaneous injection of a non-toxic aseptic irritant into the subarachnoid space and of lead by vein resulted in encephalopathy. According to Camus, lead is transported as particles of lead albuminate by the leucocytes and is excreted in the meninges when they are irritated or defective Since lead is carried in the blood as the phosphate in colloidal solution, this theory of the transportation of lead is incorrect; but Camus' experimental observations are exceedingly important because they demonstrate the relation between the mental manifestations of lead poisoning and the pathological physiology of the meninges Although there is general agreement that the choroid plexus ordinarily keeps toxins out of the cerebro-spinal fluid, certain pathological variations make possible the "secretion" or passage of toxins Ducrot and Gautrelet (103) demonstrated that methyl violet injected into the blood soon appears in the spinal fluid, and therefore concluded that this dye temporarily paralyses the choroid epithelium In cases of jaundice they observed that after methyl violet injections bile passes very easily into the spinal fluid Also Mestrezat (302) found that in cases of meningitis the choroid plexus, which is ordinarily impermeable to sodium nitrate, permits this salt to pass quite readily In fact, Levinson (259) demonstrated that when such substances as urea, chloride, and sugar are retained in the blood, their concentration in the spinal fluid increases proportionally There is, therefore, sufficient evidence of variations in the permeability of the choroid epithelium during pathological conditions to suggest the possibility that such a mechanism may operate to produce lead encephalopathy.

Because patients suffering from encephalopathy are very violent, it is difficult to examine them completely, especially to perform lumbar punctures Mosny and Malloizel (315) (316) have, however, been able to make routine lumbar taps and have found that the fluid is always under increased pressure and contains an increased number of lymphocytes (the count averaging 100 per cubic millimeter) These evidences of meningeal irritation during encephalopathy have also

been found by Norton (332), Boveri (50), and Troisier (466) who, in addition, noted that the globulin of the fluid was increased Mass (283) described a case, which he called serous meningitis complicating lead poisoning, in which the great increase in cerebro-spinal fluid produced a marked hydrocephalus Mosny divides lead meningo-encephalopathy into four classes—acute, subacute, mild, and latent—and believes that he can distinguish between them by the spinal fluid cell count In the last few years several cases of lead meningitis have been reported in small children most of whom ingested the lead by eating the paint on their beds, or from lead compounds on their mother's skin (462) (449) (451) In all of these the spinal fluid showed definite signs of meningeal irritation As might be expected with such small quantities of fluid as can ordinarily be obtained, tests for lead were negative It is therefore interesting to note that 0 08 mgm of lead were recovered from 80 cc of cerebro-spinal fluid taken from one of our patients with lead encephalopathy

This discussion indicates that in so called lead encephalopathy the meninges are primarily involved, or in other words that the disease is really a meningopathy But it is important to remember that the subarachnoid space is continuous with the tissues of the brain and with the nerves through the perineural lymph spaces (51) and that not only lead but also any pathological products formed by the action of lead on the meninges may pass along this route Therefore the brain may be involved through the passage of cerebro-spinal fluid from the sheaths about the vessels to the perineural spaces Thus, some encephalopathy may occur, and there is a possibility that lead amblyopia may be explained by a similar mechanism (158) One of the most interesting phases of this whole problem is the question of how lead can pass from the blood into the cerebro-spinal fluid Further investigation is necessary to determine what physiological and pathological conditions render the meninges susceptible to chemical toxins circulating in the blood

Part IV Clinical

XVII GENERAL CLINICAL DESCRIPTION AND DIAGNOSIS

Lead poisoning is characterized by several clear-cut and typical signs and symptoms directly referable to the absorption and action

of lead salts It is striking that there is an enormous variation in the immunity of different individuals to intoxication No test is now known which demonstrates before exposure the degree of susceptibility, but by careful and frequent medical examinations of exposed individuals it is possible to recognize susceptibility before intoxication has really developed.

There are, however, several factors which tend to reduce immunity. Certain races seem to be predisposed to lead poisoning That negroes are particularly susceptible has been stated by Edsall (106) and also pointed out to us by several physicians in white lead factories where negro labor is used. Sex and age also influence immunity Children are said to be more easily poisoned than adults (337), and the prevalence of sterility and the frequent occurrence of abortion among women exposed to lead is evidence of their greater susceptibility (25). The numerous references to the rôle of the general bodily condition in determining individual susceptibility and immunity emphasizes the importance of this factor In his book, Oliver (page 207) states that "poverty and general deprivation predispose to plumbism", while Edsall reports that "any pre-existing disease that reduces the resistance, perhaps chronic renal trouble especially, increases the liability to attack " "Any sudden unusual exposure, a drinking bout or an infection such as influenza" may produce acute symptoms (421) And finally, previous attacks of lead poisoning facilitate the later development of symptoms, usually similar to those which appeared originally but sometimes of quite different type For instance, both colic and wrist drop may recur several times or a new manifestation such as encephalopathy may develop (421)

Acute lead poisoning Although lead poisoning is generally considered to be of two types—acute and chronic—there is much confusion about the exact line of demarkation between them, for the signs and symptoms characteristic of the strictly acute type also may appear during crises in the chronic form of the disease The distinction should be recognized, however, which restricts the term acute lead poisoning to the intoxication resulting from sudden absorption of relatively large quantities of lead or to the disturbances which are rarely seen to follow brief exposure The differentiation is most clearly demonstrated in experimental animals Indeed, Straub

(447) states that in animals acute lead poisoning is a physiological experiment, chronic poisoning a disease After the rapid absorption of large amounts of lead the characteristic acute signs and symptoms are vomiting, gastro-intestinal irritation manifested by pain, diarrhea, and complete loss of appetite, general weakness, and a marked dehydration which may result in collapse or death In some cases destruction of large numbers of red blood cells in the peripheral circulation results in acute anemia and even occasionally in true hemoglobinuria Ockerblad (334) has recently reported a case in which the ingestion of 1 2 grams of lead acetate was followed by the appearance of a lead line around the teeth, stippling of red cells, marked hematuria, epigastric pain, and general malaise In the literature are to be found descriptions of mild nervous disturbances or even of convulsions and coma following sudden absorption of lead Stewart (441) states that five patients who had eaten bread colored with lead chromate died with convulsions, and Orfila (344) observed several cases of acute poisoning characterized by severe colic and coma The signs and symptoms of acute plumbism in very susceptible individuals who have been exposed to lead for brief periods are those more commonly associated with the chronic form of the disease The acute type may therefore be recognized, but lead poisoning is usually chronic as it is most frequently caused by gradual accumulation and storage of lead in the body The sudden development of acute disturbances, however, may be similar in both types of the disease

Chronic lead poisoning To define early or mild chronic lead poisoning accurately is extremely difficult and necessitates clear differentiation between absorption, damage to body tissues, and intoxication Evidence of absorption is the presence of lead in the excreta and the appearance of a lead line in the gums While neither the presence of lead in the urine and feces, nor the discoloration of the gums demonstrates in itself that there has been any injury to the organism, both signify that lead has been absorbed

Damage to body tissues may become manifest in many different ways Lead may cause the early development of arteriosclerosis, or chronic nephritis as well as other degenerative changes which bear no

apparent relation to lead poisoning This renders diagnosis very difficult. The rate of incidence of these pathological conditions is not clear, for information has been based chiefly upon poor industrial statistics

The more severe types of intoxication by lead are very easy to recognize, but the mild manifestations are so protean in character and develop so irregularly that differentiation between absorption and true intoxication is often nearly impossible Consequently, diagnosis must depend chiefly upon the number and intensity of the signs and symptoms.

For certain diagnosis of early chronic poisoning, it is usually essential that at least one typical sign of *absorption* be present, and also two distinct signs of a *generalized intoxication* by lead There are four signs which most frequently appear during the early stage of the disease· (*a*) the ashen color of the skin, (*b*) the lead line on the gums, (*c*) stippling of the red blood cells, and (*d*) a mild secondary anemia or such other evidence of rapid peripheral destruction of blood as an increased quantity of blood pigment in the plasma or of hematoporphyrin in the urine

Pallor of skin Probably the most constant of the early signs is the ashen lead color of the face and pallor of the lips Examination of the blood does not confirm the suggestion that this is caused by severe anemia alone, and Koelsch (238) believes that it is partly due to the action of lead on the skin capillaries The exact cause, however, is not really known Schmidt (400) agrees with Teleky (456) that this greyish pallor is very characteristic of lead poisoning, but thinks that intoxication may occur without it a point of view which our experience confirms.

Lead line As previously noted the so-called lead or Burtonian line was first described by Grisolle in 1835 (178), by Burton in 1839 (59) and mentioned at about the same time by Tanquerel (453) It consists of fine granules of pigment situated within the tissue of the gums about a millimeter from the border of the teeth By insertion of a piece of paper under the gum, its location within the tissues and its punctate character can be readily demonstrated. Because the discoloration is sometimes very faint, the line may often be found only

by careful examination of the margin of the gums with a hand lens after all pus and detritus have been wiped away Typically it appears around infected or pyorrheic teeth, but occasionally may be found in the buccal surfaces of the mucous membrane, i e , on the inside of the lips or cheeks near decaying teeth, and frequently at autopsy in the mucosa of the colon and lower portion of the small intestine It is stated that similar pigmentation may be caused by copper, bismuth, and silver, but that its localization is quite different

Although the line may appear when exposure to lead has been very brief, it usually persists for several months after exposure has ceased, indeed, Teleky (456) reports that he has seen it eleven months after the cessation of exposure, and in one of our cases the coloration was evident for seven months when absorption of lead was prevented In spite of the fact that the pigment lies actually within the tissues and cannot be rubbed off, careful cleansing of the mouth and teeth eliminates the line in large measure We have never observed a recurrence of the line after it disappeared during the course of treatment, but in one patient suffering from acute plumbism, it did develop under the regular hospital regime without medication Fagge (124), however, reports that in three cases of chronic plumbism, the administration of potassium iodide was followed by the appearance of the lead line.

The clinical significance of this sign should be properly recognized It is an indication of absorption only, and not of intoxication, but may almost always be found during even slight poisoning Its intensity and size provide a rough index of the duration and severity of exposure to lead, though it must be remembered that pyorrhea and dirty teeth markedly increase the rate of its development

Stippling of the red blood cells Another sign to which much importance has been attached is the appearance of stippling in the erythrocytes Our experience as well as that of some other writers, including Schoenfeld (403), is that stippling is the most reliable of the early, rather indefinite signs and symptoms of lead intoxication Although in many cases this basophilic granulation of the red cells is clear evidence of the presence of lead within the body, certain factors must be considered before assigning diagnostic value to the

stippling Even normal blood contains a very few stippled red cells; while during some chronic diseases, such as pernicious anemia and the leukemias (176), and also in certain diseases of infancy including pneumonia (58), they are very frequently observed (see section XI). Consequently before stippling can be considered a sign of plumbism, the possibility of other disturbances must be ruled out, and the proportion of stippled cells must be large enough to show that the blood is really abnormal Schmidt (400) has suggested that at least 100 cells in every million must be stippled before any diagnostic value can be assigned to the change Our repeated daily examinations of the blood during plumbism confirm the observation of Meyer and Speroni (303) that the granulation varies markedly—it may be very intense one day and practically absent the next It is therefore important to base diagnosis on more than one observation and to prepare slides with great care (For methods see page 131)

Although experience has proved that stippling is one of the most valuable of the early signs of lead intoxication, its exact significance is not known. Teleky (456) states that marked granulation is an index of the rapid absorption of lead, but the observation of marked stippling in one of our cases nine months after exposure contradicts his view and suggests that probably the red cells become stippled when lead is circulating through the organism and can affect both blood cells and body tissues Therefore, whenever it appears in large numbers of cells, further exposure to lead should be prevented immediately Both Teleky and Schoenfeld (457) report that stippling is more intense in early than in late severe plumbism, and several industrial writers confirm their belief by stating that it cannot be found in the blood of men who have been exposed to lead for long periods

Anemia Associated with stippling in the blood as one of the very early signs of the disease, is a constant mild secondary anemia The mechanism by which this is produced has already been fully described (page 133). The reduction in the number of red cells is not extremely great, and it is rare to find less than three million per cubic millimeter. The microscopic appearance of the blood is that of typical secondary anemia except that some of the red cells contain

basophilic stippling Evidence of regeneration of blood cells is manifested in early cases of intoxication by the increased number of reticulated cells As the marked peripheral blood destruction increases, the amount of blood pigments in the serum dilution test is high and the quantity of pigments in the bile is often increased The increased resistance of red blood cells to hypotonic salt solutions has been described by Malassez (275) and von Liebermann (266), and their observation has been confirmed by Orbán (343), and in this laboratory (15), but this is not usually sufficiently striking to be of much clinical aid in differential diagnosis

Lead is said to produce a relative lymphocytosis, so that though the total number of white blood cells is not increased, the percentage of lymphocytes in the blood is raised (497) (422) Our differential counts, however, have not demonstrated this strikingly, for the average differential count has demonstrated about 28 per cent mononuclear cells

Jaundice The face and sclera suggest by their slight yellow tint that the intoxication has involved the liver (362) But the coloration is probaby due to an increase in the quantity of blood pigment in the plasma because of rapid destruction of red blood cells Evidence of this is found in observations by Dr Chester Jones (229) of increased pigments in the plasma and bile It is true that Potain insisted that the liver diminished in size during lead colic, but other observers have explained the diminished liver dulness by dilatation of the intestines Tanquerel (453) recognized two types of jaundice during lead poisoning Before 1840 he made a differentiation between the two types by the statement that one was due to "extravasation of bile from its usual reservoir," and the other to "a direct change of the blood by lead" He saw only 51 cases of true jaundice in 1217 cases of lead colic

Hematoporphyrin In Germany many writers have considered the presence of hematoporphyrin in the urine an evidence of lead poisoning to which as much importance can be assigned as to stippling of the blood cells That it does appear in the urine has been fairly well ascertained (456), but its true significance seems to be a matter of debate

Gerbis (155) recognizes it as an important sign but believes that it cannot be used as certain evidence, for from 195 cases of lead poisoning he obtained the following data:

DEGREE OF STIPPLING OF BLOOD	NUMBER OF CASES	
	Showing hematoporphyrin	Not showing hematoporphyrin
Slight	5	13
Marked	12	4
Very marked	6	5

He does, nevertheless, consider that hematoporphyrin appears more constantly in the urine than does stippling in the blood, and gives data demonstrating that neither of these signs occurs in all cases In six patients he could find neither stippling nor hematoporphyrin in spite of other good clinical evidence of lead poisoning Curschman, quoted by Telekv (456), has also obtained data regarding its occurrence He found that it was present in the urine of 30 per cent of a group of subjectively normal individuals who had been exposed to lead In 40 per cent of these positive cases it finally disappeared, in 10 per cent it was still present when observations were stopped, and in the other 50 per cent definite symptoms of lead poisoning developed Unlike Gerbis, Teleky, and Schmidt, however, Curschman is reported to believe that as much reliance cannot be placed upon hematoporphyrin in the urine as upon stippling of the red blood cells And Schmidt (400) states that hematoporphyrin "is found in other diseases, as in rheumatism, heart failure with stasis, etc ," while Schoenfeld and Koelsch (238) have not been able to demonstrate that it appears constantly in the urine during plumbism

Excretion of lead The appearance of lead in the excreta is considered by many an early indication of lead poisoning Murgia (323), for instance, states that it is the earliest and most constant sign Too much weight should not be placed upon the presence of lead in the feces during exposure, for in analyzing these there is no means of distinguishing between lead which has been ingested and immediately excreted, and lead which has been actually absorbed In the urine, on the other hand, lead is present only after absorption into the circulation. During exposure, lead is not always excreted,

for all that enters the organism may be retained in the tissues Consequently, absence of lead in the excreta does not eliminate the possibility of exposure, and the presence of lead does not necessarily signify intoxication The proportion of lead excreted by the two routes varies greatly The quantity in the urine remains quite constant (see figs 20, 21 and 22) even during medication, while that in the feces may become very large

After exposure to lead has ceased, elimination in the excreta, especially in the feces, may continue for a very long time We have found lead in the feces of a workman three years after he left his hazardous trade, and Oliver (337) has found it in the urine eleven years after removal from the hazard Even when the excreta contain lead for such long periods, there is no justification for assuming that lead has produced deleterious effects These, of course, should be looked for, but lead may and often does enter and leave the body without causing intoxication, and may at one time or another be excreted by any one exposed to lead

Other signs The other signs which commonly appear in the early stages of lead poisoning are not as constant as those already mentioned A fine tremor may frequently be seen in early as well as in advanced cases Although it is often not very striking, it is located in the muscles of the hand, the tongue, or face particularly around the eyes, and the eyelid, and is sometimes manifested as fibrillary twitching It is easily confused with the tremors found in many other diseases, and cannot, therefore, possess much diagnostic importance, but its frequent appearance deserves notice

Another early sign of plumbism often mentioned by industrial physicians is high blood pressure In our cases this could not be observed, and it hardly seems probable that hypertension can be of any great value in the diagnosis of early lead poisoning

Teleky (158) considers a diminished power to extend the wrists a very common early sign of the disease

Associated with these signs are many symptoms, such as headache, indefinite weakness and lassitude, lack of appetite accompanied by loss of weight, nausea, constipation, metallic taste in the mouth,

and halitosis. These signs and symptoms together are characteristic of the early stages of lead poisoning

Toxic episodes. Workmen exposed to lead may frequently show the early signs for a long time without any acute developments Then suddenly one of the toxic episodes (called "symptomes episodiques" by Vibert (477)), may be precipitated These are colic, palsy, encephalopathy, optical disturbances, and so-called lead arthralgia. Why some people develop these after only a few weeks of exposure to lead, while others show nothing for many years and then suddenly develop colic or wrist drop, is difficult to understand But immunity is always relative, and may be broken down by several factors which influence the general physical condition Oliver (337) mentions undernourishment and poor diet, and Edsall (106) chronic diseases, particularly nephritis, as predisposing influences Likewise, an acute infection, such as pneumonia or influenza, is very apt to produce acute symptoms, as Marvin Shie (421) and also Dr Brockway of Brooklyn[3] observed during the influenza epidemic of 1918. Delearde and Du Bois (93) and others have also noted that lead colic usually follows a few days of alcoholic excess, and Shufflebotham (427) has seen acute symptoms develop in a lead worker after injury

Once an acute symptom has appeared, attacks follow with increasing ease Lead colic, for instance, tends to recur, and muscles which have been paralyzed or weakened usually suffer the first injury on re-exposure. But succeeding attacks are not necessarily similar in character, and the acute symptoms of a second attack may be entirely different from those of the first Marvin Shie describes cases in which wrist drop followed re-exposure to lead although muscular weakness had not been a previous symptom. Even without a second absorption of lead from external sources, attacks of lead poisoning may recur

Colic. The most common and dramatic of the acute manifestations of plumbism is colic Tanquerel reports that of 1493 cases of lead poisoning, 1207 suffered from this symptom—ten times as many as from palsy, and seventeen times as many as from encephalopathy Consequently, it is usually the first acute episode to develop

To most observers the term colic implies the severe intestinal pain

[3] Personal communication

which so very frequently follows absorption of lead, but this is only a restricted use of the word Since any of the smooth muscles of the body may be affected by lead, colic may be of various types At times spasms involve the muscles of the bladder and prevent urination Tanquerel describes involvement of the muscles of the testicle, and also spasm of the uterine and vaginal muscles which produces pain similar to that of child-birth In giving enemas, we have observed contraction of the rectal sphincter on insertion of the catheter By far the most common manifestation, however, is that which involves the intestinal tract

While suffering from colic, patients are cold, pale, and often drenched with perspiration They commonly bend over and writhe about the bed in intense pain Between attacks they are usually very restless, probably because of the sense of constant pressure in the abdomen which persists even between the intermittent spasms The pain is of a tearing or griping nature and its intensity varies markedly It occurs intermittently but with no apparent regularity —attacks are separated by intervals of only a few minutes or several hours Although usually below the umbilicus, the discomfort shifts from one part of the abdomen to another, and patients often show its location characteristically by spreading both hands widely over the lower part of the abdomen Sometimes local areas of hyperesthesia which migrate are present

Acute attacks of colic are nearly invariably preceded by several days of obstinate constipation During both this preliminary period and the attack itself, there is often a desire to defecate, but there is usually complete inability to pass anything except sometimes a small quantity of mucus The vomiting, which occurs quite frequently with the onset of pain, may be of very brief duration or may persist until the other acute symptoms subside The vomitus usually contains bile, is green and frothy in appearance, and small in amount Excretion of urine is decreased

The appearance of the abdomen during attacks of colic is quite characteristic It is rarely distended, but is usually scaphoid in shape and held tensely and rigidly Between attacks of pain the abdomen has a generalized doughy resistance and there is no true abdominal spasm

In duration as well as time of onset, colic is very variable Of 31 cases which Tanquerel des Planches observed in the hospital without treatment, one recovered on the fourth day, one on the seventh, and eleven between the eighth and twelfth In fifteen, colic persisted until treatment was begun after more than twelve days in the hospital Brouardel (55) states that colic usually yields to treatment within eight days, but that if neglected it may continue for from two to five or six weeks Usually, however, in our experience treatment brings relief within two days

Differential diagnosis Differentiation between lead colic and the abdominal inflammations which require prompt surgical treatment is often very difficult in spite of several marked characteristics The history of exposure as well as other evidence of lead poisoning help markedly in establishing diagnosis During true lead colic, the temperature rarely rises more than one degree The number of white cells in the blood remains nearly normal, and any increase is caused by mononuclear rather than by polymorphonuclear leucocytes The pulse is often remarkably slow There is also no real muscular spasm, though the abdominal wall may feel generally doughy and resistant There is also the usual history of marked constipation

Prognosis. In itself lead colic is not dangerous In fact, Lewin is said by Brouardel (55) to have collected data from six thousand cases which show that in this number death resulted from colic only once. Recovery is always fairly prompt and complete though constipation tends to persist As has already been mentioned, a single acute attack of lead poisoning renders an individual more susceptible to future attacks Distributed through the literature are histories of cases in which, without further exposure, colic has recurred many years after the primary exposure had ceased This usually occurred after severe illness or alcoholic debauch or after the administration of potassium iodide

Lead palsy. Another striking but less frequent manifestation of lead poisoning is palsy. This was recognized by the ancients and as early as 1656 Stockhusen (444) wrote a good treatise localizing the lesion. Many others, particularly De Haen (90) in 1771, studied it well clinically, but Tanquerel des Planches (453) and Duchesne

(102) in the early part of the nineteenth century gave the most satisfactory clinical description

Probably when these investigators lived, lead palsy was a far more common manifestation of plumbism than it is at present, because exposure to lead was much more severe than now But in only 13 per cent of the cases of lead poisoning seen by Tanquerel was it observed

Prodromal signs Usually before the typical symptoms of this type of lead poisoning become evident, prodromal signs appear Colic, for instance, or other acute manifestations may occur, or a general lassitude accompanied by painful cramps or tremor of the muscles which are later involved Sometimes formication, numbness or a dull ache, and very rarely hyperesthesia, develop in the injured regions "Even when sensibility is in the normal state, patients experience a sensation of fatigue and heaviness in the paralyzed parts, principally in the articulations connected with affected muscles" (453)

The manifestations of palsy are very varied in character and severity Muscular weakness may be the only sign of injury or motion may be completely lost The appearance of symptoms may be quite gradual, or very rapid as in the cases reported by Schlapp (399) and by Windscheid (495) when paralysis developed suddenly and without warning after trauma Shie (421) states that this may also occur upon re exposure to lead

The onset of paralysis does not appear to be closely related to length of exposure In 102 cases observed by Tanquerel in industry, palsy developed in 9 during the first month of work, in 14 during the second, in 36 within the first two years, in 32 after ten years of exposure, in 13 after twenty years, and in one after exposure had continued for fifty-two years Thus neuro muscular lesions may develop at almost any stage of plumbism

Perhaps the most satisfactory description of the different types of lead palsy has been given by Dejerine-Klumpke (92) in her book published in 1889 By far the most common form is the well known wrist drop or antibrachial paralysis which may be limited to the extensor muscles of the middle and ring fingers In one case which we have observed, in spite of continued exposure, such paralysis did

of great interest, for in their work file cutters not only use particularly the muscles mentioned above but place the greatest strain upon the left hand

Although not a frequent occurrence, paralysis of the lower limbs does develop during lead poisoning In 97 cases of palsy Tanquerel observed it 15 times, and Thomas (461) reports that it occurred in 4 of his 31 cases Of these 19, only 5 patients suffered from paralysis of the lower limbs without involvement of other parts of the body Although sometimes generalized, this type of paralysis commonly develops in regions analogous to those affected by wrist drop—in the lateral peroneal muscles, the common extensor of the toes, and the extensor of the large toe The other muscles of the leg, however, retain their normal strength

In addition to these characteristic manifestations of lead palsy, certain more generalized disturbances may be observed during chronic poisoning Paralysis may spread from the leg or hand until by involving the diaphragm or larynx it causes death There are other generalized cord lesions which have been attributed to the action of lead Putnam (370) reports several cases, which were proved to be a manifestation of plumbism by the presence of lead in the urine, which looked like lateral sclerosis, and those of several other investigators bore a strong resemblance to diffuse anterior poliomyelitis (369) A few cases of generalized paralysis, amyotrophic lateral sclerosis, and spastic paraplegia have been reported (461) (419) (109) (28) (318) (270) (311) which seem probably to be caused by lead Putnam mentions that in five cases of pseudo-tabes, with incoordination and ataxia, in which lead was excreted in the urine, prompt recovery followed treatment Recently, Krafczyk (241) has reported an interesting case of cerebellar ataxia caused by lead The red blood cells of his patient were stippled, and anemia, nystagmus, and general weakness with marked ataxia were noticeable The muscles most affected were those most fatigued In most of the reported cases of generalized cord lesions, however, sufficient evidence of lead poisoning is lacking

A theory explaining the localization of lead paralysis was first clearly developed by Edinger (105) Manouvrier (280) noted in 1874 that in a series of cases, palsy was most evident in the hand

most used, a fact which he explained by direct absorption through the skin. Two years later Remak (380) pointed out that the muscle groups functionally related are involved in characteristic order irrespective of their innervation Ten years later Moebius (312) reported that in file cutters, palsy usually develops in the thenar and hypothenar eminences of the left hand—muscles which are greatly fatigued in their work, and by 1908 Edinger (105) had fully developed his theory of the appearance of palsy in those muscles which are most used This was followed in the next year by additional evidence from Teleky (455). This whole theory is very important and is but little disputed at present The experimental data upon which it is based have already been discussed

There are certain features of lead paralysis which facilitate diagnosis and should always be kept in mind It is almost never associated with fever, and this fact, as well as absence of stiffness in the neck, localization in the most used muscles, and the occurrence of some of the various other signs of lead poisoning, must sometimes be the basis of differentiation between lead palsy and poliomyelitis. Progressive muscular atrophy, amyotrophic lateral sclerosis, cervical rib, arsenic or alcoholic neuritis, and similar conditions must also be differentiated

If further absorption of lead is prevented as soon as paralysis develops, complete recovery usually occurs, although it is often very slow. Teleky states that continued exposure retards or prevents improvement Wrist drop which has persisted for very long periods may gradually disappear almost entirely It is interesting that the muscles first affected by lead palsy are the last to recover

Lead encephalopathy. In almost every case of plumbism there is a change in the mental attitude which may be so slight as to be overlooked entirely, or may consist in general sluggishness and dulness, with poor memory, inability to concentrate, and a tendency towards restlessness and irritability Although in some cases encephalopathy may develop suddenly and without warning, these same symptoms in a slightly exaggerated form often precede the onset of severe disturbances There are in addition, certain other prodromata described by Grisolle (179) and Tanquerel which deserve mention. Any signs or symptoms of lead poisoning, no matter of what charac-

ter, may appear before encephalopathy becomes manifest, and restless sleep, general nervousness, mental depression, persistent headache, vertigo, faintness, transient paralysis, tremor of the hands, and tingling sensations may be noticeable shortly before the onset of general convulsions

Fortunately, as industrial conditions have improved with fuller knowledge of lead poisoning, the incidence of lead encephalopathy has progressively decreased until this severe manifestation of plumbism has become infrequent Most of the ancient writers speak of the frequent occurrence of epileptiform convulsions, but no good clinical description of lead encephalopathy appeared until the time of Grisolle He published an article in 1836 in which he reports his observations and divides lead encephalopathy into three groups—convulsive, comatose, and delirious He studied 29 cases of true encephalopathy, and describes very fully ten of these "accidents cérébraux" As these observations were so complete, Tanquerel's description of 72 cases added very little to an understanding of the nature of this type of lead poisoning In spite of the many investigations which have been made since that time (489) (460), the only outstanding new clinical contribution is the differentiation between syphilitic and lead encephalopathy

Of the three forms in which this type of lead poisoning is manifested, Grisolle found that the convulsive or epileptiform is the most common and serious It occurred in fifteen of his twenty-nine cases and proved fatal in eleven Six times the first evidence of the trouble was a severe convulsion The nature of these attacks is very much like that of an epileptic seizure with clonic contraction of the whole body, frothing at the mouth, incontinence of urine and feces, followed by coma Stewart (442) describes an epidemic of encephalopathy which occurred in a family after eating buns colored with lead chromate (approximately 2 grains in each bun) With much difficulty he was able to elicit a history of colic and pain, accompanied by the vomiting of greenish material, before the sudden onset of convulsions In these cases, attacks which resembled epilepsy recurred frequently and proved fatal in four of the six children The literature on this type of epileptic encephalopathy has been well summarized by Badie (23) and Thieme (460) who have also collected many case his-

tories of epileptic seizures of all degrees of severity. In Boston within the last two years, we have seen four cases, three of which died The other two types of encephalopathy mentioned by Grisolle are much less frequent and are really not distinct Delirium occurred in seven of his twenty-nine cases, and in three which we observed Six of these ten cases died. In only five of Grisolle's cases did coma develop Kiernan (235) also describes a melancholic type which, when chronic, is associated with progressive mental deterioration

In animals exposed to lead either in the laboratory or in factories working with lead compounds, unmistakable signs of lead encephalopathy have often been noted Grisolle mentions this, and it is well known now among workmen that cats living around lead factories are affected with palsy and have convulsions after being exposed for some time Porak (361) reports that many of his guinea pigs died in convulsions or with palsy during experimental poisoning, and in five of our animals similar symptoms developed (four cats, one rabbit). As all of these animals manifested practically the same disturbances, the history of only one will be given here.

Cat 222 A suspension of lead carbonate was administered intratracheally to this animal three times in nineteen days Four days after the last injection there was lack of coordination When left alone the cat lay quite still with its head bent over toward the left paw If made to stand, it would start to walk with very rapid steps and would rush toward the right until it fell or hit the wall It never turned toward the left The cat lay quietly on either side, showed no rigidity in legs or paws, and no paralysis or wrist drop Although there was no nystagmus if the animal faced directly forwards, nystagmus was quite definite when the head was turned to either side, particularly toward the right On the second day of the disease severe clonic contraction developed, with the head retracted and the whole body stiffened and twitching Death occurred six days after the last injection of lead during one of these convulsions Post mortem examination showed that, although some of the lead had been deposited about the trachea, most had entered the lungs and had been deposited in the upper portion of the left lower lobe Macroscopically, the rest of the body was not remarkable No typical stippling could be demonstrated in blood smears taken before and after death, nor in any of the bone marrow cells The red count six days before death was normal. Lead was well distributed throughout most of the body but there was none in the brain and

cord Microscopic examination of the cerebellum and cord made by Dr Mella disclosed nothing remarkable except that in the cerebellum there was swelling, chromatolysis, and vacuolization of almost all the Purkinje cells Many of the nuclei were swollen, others were extruded, and the cell bodies were fragmented Only rarely were normal cells seen This was observed with the cresyl-violet stain Van Humann stain, and phosphotungstic acid stain showed nothing abnormal either in the cerebellum or in the spinal cord The meninges appeared normal

In three cats severely poisoned with lead, convulsions occurred during exercise in a treadmill, and as attacks never appeared in normal animals during similar exercise, or in these poisoned cats at other times, fatigue must have been the immediate cause

It is interesting that lead is not usually found in the spinal fluid Thus, Strong (449) reports that he was unsuccessful in an attempt to find it in the fluid obtained from an infant who died of lead meningitis, and Krafficzyk (241) found none in material taken from a patient with chronic ataxic lead poisoning Two specimens were examined in this laboratory One was taken from a man who had mild plumbism but died two years later from general paresis, and, although 50 cc of his spinal fluid was examined, no lead was found The other was from a patient with marked encephalopathy, and microchemical quantities of lead were demonstrated in 80 cc

As yet no satisfactory explanation for lead encephalopathy has been found

Traube (464) suggested that edema of the brain might be the cause of symptoms, Rosenstein (390) mentions spasm of cerebral vessels and consequent anemia as a possible mechanism, and Westphal (489) believed that lead acts directly on the tissue of the brain, or establishes a condition of uremia upon which the symptoms depend But all of these suggestions are based purely upon theory and no data have been obtained from which a satisfactory explanation may be derived

On account of its similarity to other types of disease, differential diagnosis of lead encephalopathy is very difficult It may simulate tumors and syphilis of the central nervous system, cerebral hemorrhage, epilepsy, chronic alcoholism, uremia, eclampsia, hysteria, and even neurasthenic disturbances, and great care must therefore

be employed in differentiation By the appearance of certain signs, however, it is usually possible to distinguish the disturbances occasioned by lead The lead line, stippling of the red blood cells, anemia, lead in the excreta, or colic may frequently be observed On lumbar puncture, an increase in the pressure of the spinal fluid with an increase in the number of cells, especially of lymphocytes, is characteristic of lead encephalopathy, while the Wassermann test is negative

Although statistics show that a large percentage of patients with lead encephalopathy die, it is dangerous to give actual figures, for in different reports the mortality rate varies from 25 to 75 per cent If patients survive, their mentality sometimes remains distinctly changed They are apt to have melancholia and delusions, or a poor memory and mental sluggishness, and, occasionally, epileptiform convulsions

Optic lesions due to lead. That lead causes neuritis of the eye and palsy of the eye muscles has long been known Tanquerel found but twelve eye lesions in his series and thought this complication very unusual, a point of view generally held, as for instance by de Schweinitz (410). And yet certain authors, such as Lockhardt Gibson (157) (158) of Australia, who in fifteen years saw 54 cases of ocular neuritis due to lead, insist that the effects of lead on the eye are not very rare Of the various recent publications on the subject the articles by Lewin (261) in 1904 and Lockhardt Gibson are perhaps the most interesting The lesions of the eye take various forms Palsies and cramps of the various individual eye muscles, as well as blindness due to optic atrophy, and inflammation of the retina with the subsequent development of atrophy also occur. Hemianopsia occasionally develops Lewin says also that corneal opacities are sometimes due to lead

The lesions of the optic nerve, however, are interesting because they are unique, inasmuch as they involve a nerve of special sense Several explanations have been advanced for this, for instance, Weiser (485) thinks that the action of lead may be due to a change in the blood vessels in the retina which causes hemorrhagic retinitis Lockhardt Gibson, however, claims that nearly all the cases of toxic amblyopia due to lead have been associated with signs of encephalopathy or

of increased pressure in the spinal cord, and that it is usual for the optic disc to swell as much as three diopters This is possibly the reason that a poison which does not attack sensory nerves should affect the optic nerve This view is not generally held, however, and primary optic atrophy due to lead is said to occur rarely

Gout That pain in the muscles and joints is not infrequently associated with lead intoxication was first pointed out by Tanquerel (453) He gives a very full and satisfactory description of the so-called lead arthralgia, which appears in the joints and muscles of the extremities—more severely in the flexors than the extensors, and in the legs than in the arms This pain has been repeatedly mentioned by other workers, and recently Wright (497) has stated that "indefinite pain in the muscles is common," and indeed that "pain in the joints, especially of the knees and elbows, is an important and usually early symptom" of lead poisoning He also says that "Bursitis is not infrequent Numerous cases of elbow pain in connection with lead poisoning have been studied In a few instances the pain was closely localized to the external epicondyle of the humerus, but the majority showed tenderness on pressure over the head of the radius, the site of a bursa described by Dr Robert Osgood"

Saturnine gout has also been described Although Skagge (432) is said to have observed gout in lead workers in 1764, and Garrod (153) in 1857, pointed out that it was quite common among lead workers in England, gout has not been considered a usual complication of lead poisoning Hubner (221) states that it is really not an uncommon symptom in older workmen and that it may even be seen in young men His description is that of the typical gout which most often affects only the large toe but sometimes spreads to other joints Brouardel (56) also states that he has seen six cases of gout in lead workers It may, therefore, be possible that lead intoxication and a gouty diathesis are associated, although we have not observed it

Late manifestations Generalized arteriosclerosis is often seen in individuals who have long been exposed to lead, and chronic nephritis of arteriosclerotic origin may also be observed That these result from the action of lead cannot be proved, but their incidence in lead

workers is high The symptoms and signs do not differ from those usually seen in arteriosclerosis due to other causes

Summary. This chapter briefly describes the principal clinical manifestations of lead intoxication and the acute toxic episodes which develop during the disease

XVIII STANDARDS OF DIAGNOSIS

Individual opinion differs widely about the data necessary for definite diagnosis of lead poisoning Some physicians believe that diagnosis cannot be certain without the development of at least one of the toxic episodes, while others consider such very meagre evidence as constipation and slight stippling of the red blood cells sufficient Our experience seems to indicate, however, that the intensity and number of the signs and symptoms, the interval between their appearance and the beginning of exposure, and evidence of any injury to body tissues which may be directly attributed to lead, are all factors which must be considered in the establishment of diagnosis

Great variation in the appearance of these usual signs and symptoms is common Indeed, in some individuals marked absorption may be indicated by a single sign—a heavy lead line, for instance—without any subjective symptoms of intoxication, while in others, marked symptoms obviously referable to lead may develop although the usual early signs are entirely absent Quite rarely in very susceptible individuals it is said that such an isolated manifestation as colic may be the only indication of poisoning But such cases as this are not common and, in general, diagnosis of *intoxication* by lead as distinguished from *absorption* must be based upon the appearance of both subjective symptoms and objective signs

A good standard of diagnosis has been suggested by Newman, McConnell, Spencer, and Phillips (330) in their book entitled *Lead Poisoning in the Pottery Trades* They lay great stress on the determination of known or suspected exposure to lead and on personal history and physical examination as means of eliminating conditions which might simulate symptoms of lead poisoning The recognized signs and symptoms they divide into two groups The first of major symptoms; the second of more generalized and less acute manifestations In their opinion a diagnosis of lead poisoning cannot be made

unless at least two of the systems in Group A are affected and the numerous minor symptoms of true lead intoxication are present If, however, only one sign included in Group A may be found, at least two more must be present from separate divisions of Group B to establish early diagnosis When only signs from Group B appear, at least three from separate divisions are necessary before a tentative diagnosis of lead poisoning may be considered

Group A

A General appearance
  Marked pallor and profound anemia
B Digestive system
  Colic
  Obstinate constipation
C Muscular system
  Muscular incoordination
D Nervous system
  Peripheral motor paralysis of certain extensor muscles (wrist and ankle drop) and atrophy of most used set of muscles
E Vascular system
  Blood Basophilic degeneration with diminished hemoglobin
F Special organs and findings
  Gums Lead line
  Stools and urine Lead
  Miscarriage Repeated
  Liebermann's test Positive

Group B

A General appearance
  Pallor
  Anemia
  Emaciation
  Drawn expression
B Digestive system
  Loss of appetite or repugnance to food
  Breakfast anorexia
  Vomiting on eating solid food
  Sweetish or metallic taste
  Gastric disturbances
  Constipation

Pain in abdomen.
Parotitis.

C. Muscular system:
Loss of strength.
Malaise and tiring easily

D. Nervous system:
Headache.
Insomnia.
Mental lethargy.
Tremor.
Dizziness.
Convulsions
Mental affections
Encephalopathic conditions.
Arteritis.

E. Vascular system:
Arteriosclerosis.
Hypertension.

F. Special organs and findings·
Eyes. Impairment of vision, including muscular incoordination.
Joints· Various pains.
Blood: Basophilic degeneration with diminished hemoglobin

Of its sort this is a satisfactory standard. But it has the distinct disadvantage of assigning to a single sign or symptom positive or negative significance, whereas as a matter of fact there is a subtle gradation which makes the final decision a matter of judgment. For instance, there may be one stippled cell or many in a blood smear, and the lead line may be marked or evident only under a magnifying lens. In the last analysis, therefore, the early diagnosis of lead poisoning must depend upon keen judgment, and no fast rules can be established.

In order to avoid further exposure and to prevent severe intoxication it is essential that lead poisoning be at least suspected very early. Although the appearance of any one of the toxic manifestations, in an individual known to be exposed to lead, is sufficient evidence for this, definite diagnosis cannot be made without more decisive data. Thus, either a history of exposure to lead or the presence of lead in the excreta must be ascertained, and at least one of the characteristic

signs included in Group A must have developed during exposure and be associated with other distinct evidences of the disease which have also followed absorption, before diagnosis can be certain Very little weight can be placed on the mere presence of such common persistent symptoms as constipation, anorexia, headache, insomnia, etc , unless they have markedly increased since exposure, for lead workers are no more immune to the other diseases which might also cause these symptoms than are individuals who are not exposed to lead Therefore the development of distinct toxic symptoms after exposure is necessary for definite diagnosis

Toxicity It is difficult to determine even roughly the dose of lead which should be considered toxic, for the quantities eliminated without absorption from the gastro intestinal or respiratory tracts cannot be measured, and individual susceptibility varies greatly Teleky, however, is quoted in Oliver's book (page 37) as believing that ingestion of a little more than 1 mgm of lead per day for several months causes plumbism and that a daily dose of 10 mgm causes severe symptoms in a short time Legge (252) thinks that 2 mgm per day may cause chronic poisoning, while Sollmann (434) from a summary of the literature concludes that, in man, daily ingestion of 0 2 to 0 3 mgm of lead per kilo will in time produce phenomena of lead poisoning He thinks, however, that more minute doses might possibly interfere with nutrition and resistance

XIX THE TREATMENT OF LEAD POISONING

In the treatment of lead poisoning three definite problems are involved Prevention, treatment of acute symptoms, and treatment of the disease as a whole Much has been written on prevention in the last decade, chiefly by Alice Hamilton in America, Legge and Goadby and Sir Thomas Oliver in England, and by Teleky in Germany, with the result that the incidence of lead poisoning has decreased markedly A full discussion of the means used to prevent plumbism does not belong in this monograph, but it may be of interest to summarize briefly the various problems encountered in industry The following outline has, for the most part been taken from Hutton's (222) excellent review

Prevention. Exposure to lead may be prevented in two ways By devising suitable appliances and processes for manufacture, and by medical supervision of workmen. Since medical supervision can accomplish little when exposure is very severe, the first step is always to ascertain that every precaution has been taken to render factory conditions safe The very rapid absorption of lead from the respiratory tract points to dust as the most dangerous source of lead. Consequently, every effort ought to be made to substitute wet processes for those in which dry and dusty chemicals are used, to store and transport lead in air-tight receptacles, to equip machines whenever necessary with suction drafts which draw dust from the workroom, and to cover pots of molten metal and place them under a suction hood so that the lead oxide, which forms as a scum on the surface, cannot blow about Great emphasis must be placed on cleanliness, for if suction drafts become clogged, these precautions are useless Dust should never be allowed to accumulate Mechanical processes ought always to be substituted for manipulations by hand, if possible, and molten lead should not be heated above 700°C on account of vaporization. White lead ought to be prepared only by moist processes, and as Klein (236) has demonstrated paint should be rubbed down only with abrasives, such as waxed sandpaper, which can be used on moistened surfaces.

Whenever mechanical devices fail to eliminate dust, work must be so arranged that as few men as possible are exposed, and careful instruction be given as to dangers involved

Certain measures of hygiene are particularly important Workmen should wear clean clothes while at work, and always keep them quite separate from those worn at other times This requires two lockers, for if dusty clothes are hung at night where street clothes hang during the day, contamination can hardly be prevented Smoking or chewing tobacco, if the hands are at all dirty, involves the danger of ingestion of lead, and as food may also be contaminated in the same way, provision must be made for washing thoroughly, and for eating in rooms completely removed from the lead hazard Before dressing to leave the factory, workmen must be required to wash off all dust with great care

Respirators have proved an unsatisfactory means of protection

because they either make breathing difficult or fail to collect all the dust They usually fit so poorly that considerable dust enters between the edges of the mask and the cheeks Consequently, they merely give a sense of security without providing adequate protection While they may not be entirely discarded, it must always be remembered that the use of respirators does not remove responsibility for the elimination of dust in the workrooms This is the duty of every factory utilizing lead or its salts

Medical supervision should be thorough Physical examinations determine the fitness of applicants for work, and any individuals who have had repeated attacks of lead colic or other form of lead intoxication, who show a marked degree of anemia, or evidence of nephritis, or high blood pressure ought to be rejected (337) Preferably only men should be employed, and only those who are in good physical condition and who are not mentally defective During exposure to lead, employees must be carefully watched and examined every week or two in order to detect very early the development of anemia, pallor, lead line, or other symptoms of active lead intoxication The physician should be given authority to order the transfer of suspicious cases from hazardous positions, or to request a period of rest Upon re exposure great emphasis must be placed on the necessary precautions, and the factory physician should be rigorous in his supervision

The treatment of lead poisoning Before the dangers of exposure to lead were understood, and when lead salts were used for medication and to sweeten and preserve wine, the only forms of lead intoxication recognized were the "symptomes episodiques" According to the older physicians these required very drastic treatment, and many so called "specifics" came to be employed As colic is the most common manifestation of poisoning and responds most readily to treatment, more different treatments have been devised for it than for the other symptoms

The oldest and probably the most renowned is that employed by the Italian monks who took charge of the Hôpital de Charité established in Paris in 1602 by Marie de Medicis Their "specific," called "macaroni," consisted of one part of glass of antimony and two parts of sugar very carefully powdered and mixed This was given in doses of one scruple for

three or four successive days As time went on, however, treatment became more and more complicated until it consisted of the administration of violent purgatives, sedatives, and sudorifics. "C'était une méthode antique et spécifique, de laquelle il ne fallait pas même demander les raisons (453)," or as Dana translates the French, "It was a violent specific, but respectable from its antiquity" In 1836 Tanquerel (453) found that croton oil was just as effective as the more powerful traditional medication, and in treating his patients he usually employed this purgative Although Moseley (314) and later Gendrin (154) advocated the use of sulphuric acid lemonades as very effective, Tanquerel did not consider their action particularly successful in relieving colic. Frequent reference is also made in the literature to the use of nux vomica, opium, antiphlogistics, and leeches, as well as to cupping and bleeding as suitable treatment for lead colic.

The first mention of a course of treatment to aid excretion of the store of lead within the body was made by Melsens (297) in 1840 He performed experiments on animals which demonstrated that potassium iodide when used with purgatives is a very effective form of medication, and concluded that it produces in the body lead compounds which are soluble and can be readily excreted When administered to animals simultaneously with lead, he noticed that it prevented intoxication, in previously "leaded" animals, however, large doses caused fatal poisoning Although Melsens obtained excellent results in treating his patients with potassium iodide, he did not analyze the excreta to determine the exact effect of this medication, and it was not until 1853 that Parkes (352) found lead in urine after administration of potassium iodide when it had not been excreted by this route before treatment His observation was verified by Annuschat (10) in work on a dog with lead poisoning, and also by Pouchet (366) who noted that the rate of excretion is raised for only a few days, and falls promptly to the normal level In 1893 Dixon Mann (279) carried out very careful investigations of the effect of potassium iodide which led him to conclude that although this drug apparently makes patients feel more comfortable, it does not increase excretion of lead in urine

Thus, data concerning the efficacy of potassium iodide in the treatment of plumbism have been quite contradictory and further experiments were necessary to determine just how it produced its beneficial effects

We have therefore analyzed both urine and feces repeatedly in this laboratory in an effort to throw some light on the problem. Our detailed data (see section IX) show that potassium iodide defi-

nitely increases excretion of lead—on the average approximately doubles the quantity eliminated—and that this increase appears for the most part in the feces This accounts for the results reported in the literature, for previous investigators examined the urine only The increased excretion of lead justifies the use of this drug after the subsidence of acute symptoms It is best administered three times a day in doses increasing from 3 to 5 grains to the limit of tolerance During acute attacks of plumbism no such medication should, of course, be given because the development of symptoms implies the presence of toxic quantities of lead in the blood

That potassium iodide may precipitate an acute attack of lead poisoning in apparently healthy individuals long after exposure to lead has ceased, is repeatedly stated in the literature Probably many authors mention this merely as a warning, for few definite cases are cited, but Oliver (337) Fagge (124) and Lazell (250) report observations worthy of consideration Of Oliver's two cases, one is that of a woman of seventy-two, who is said to have died of "lead poisoning" after taking 7 grains of potassium iodide As the patient was suffering from acute symptoms of poisoning before treatment was begun, this case does not illustrate the point as well as Oliver's second case In this, administration of potassium iodide was promptly followed by the appearance of a lead line and wrist drop, although there had been no previous evidence of intoxication In 1876, Fagge (124) reported three cases in which a lead line developed under similar conditions Lazell (250) also has described the appearance of double foot drop, lead line, and digestive disturbances with colic in a man who had had severe lead poisoning sixteen years previously without subsequent exposure Three months before the onset of this palsy the patient had had lobar pneumonia, and potassium iodine had evidently been given to relieve persistent lung involvement Probably this tendency to promote disturbances is not restricted to potassium iodide alone, and any other treatment which liberates lead from the deposits in the body might precipitate acute symptoms by allowing too much lead to enter the blood stream (section IX)

Although so much importance has been ascribed to potassium iodide in the treatment of lead poisoning, it is not the most effective agent

for increasing excretion of lead from the body. References to the use of acid or alkalies are very few.

Gendrin (154), early in the nineteenth century, attempted to render lead salts inactive in the body by transforming them to insoluble sulphate with sulphuric acid Sabrazès (393) mentions that acute symptoms developed in a case of late chronic plumbism after inordinate drinking of "citronade", and Brouardel (56) states that sour salads will cause colic to recur. But the true significance of these observations on organic acids cannot be ascribed to the production of an acidosis

The experiments reported in section IX indicate that acids and, to a less degree, alkalies are distinctly effective in increasing the elimination of lead This is apparently due to two factors a negative calcium balance, and a distortion of the normal acid-base equilibrium of the tissues. Marvin Shie (422) has already suggested as a result of his clinical observations that metabolic disturbances probably play a noteworthy rôle in the liberation of lead

A new treatment for lead poisoning which has proved satisfactory in our cases is based upon our observations of the excretion of lead in cats and man The foundation for this is the fact that the quantity of lead excreted may be varied by distorting the calcium metabolism, and that a positive calcium balance favors storage of lead, while a negative balance tends to increase the rate of excretion. The association of active symptoms with a marked distribution of lead throughout the body—in other words, an active lead stream—indicates that when symptoms of lead intoxication are evident it is wise to immobilize lead by impeding its liberation from the bones Therefore, until an acute attack has disappeared the diet should contain much calcium in the form of milk and calcium lactate As, in our experience, the normal minimal excretion of calcium per day, when the diet is deficient in calcium, appears to be 0 235 gram, and as a quart of milk contains approximately 1 1 gram, one quart of milk and 2 grams of calcium lactate should adequately fulfill the requirements Under this treatment alone colic rapidly subsides, but it is advisable to give atropine and cathartics in addition

We have not determined the actual effect of these various medica-

tions upon the duration of colic To do this satisfactorily would require a very large number of observations, and there are in the literature practically no reports of the duration of untreated colic except the 31 cases of Tanquerel (453) In these, the average duration without relief was more than ten days Nearly all of our patients have been practically relieved within forty-eight hours, and only two had definite cramps for a period of eight days We have not observed colic without treatment nor with catharsis alone, but certainly merely a high calcium intake and catharsis cause it to subside rapidly In the three cases of encephalopathy in which this treatment was used, striking recovery was very prompt

Not until acute symptoms have entirely subsided should "deleading" be attempted This is accomplished by maintaining a negative calcium balance and changing the hydrogen ion concentration of the blood (For the basis of this treatment, see discussion on page 96) To do this, either acid should be administered with a diet which contains very little calcium, or alkalies should be given alone During this course of treatment, the diet contrasts sharply with that advocated while definite symptoms are manifest Milk, eggs, green vegetables, and many fruits are omitted entirely In our experience the following diet has proved satisfactory

Meat
Liver
Potato
Rice
Tomatoes } cooked without milk
Canned corn
Bananas
Apples (peeled)
Tea and coffee without milk
Butter fat (prepared by melting butter in hot water and skimming off the butter fat)
Bread (prepared without milk, such as salt-free nephritic bread or soda bicarbonate biscuits or crackers)
Sugar—salt—pepper

Under this regime, acids mobilize lead effectually Phosphoric acid has proved the most useful, although it is rather unpleasant to

take We have found that when mixed with water and sweetened it tastes somewhat like lemonade and may be taken through a straw without discomfort If the dose proves very disagreeable, a little gin or whiskey makes it more palatable By rinsing out the mouth with water or a solution of sodium bicarbonate, any excess acid is easily removed and injury to the teeth avoided Doses of 20 cc of the dilute acid should be given in a glass of water every hour about ten times daily for several weeks If acidosis becomes too severe, loss of appetite, headache, and general malaise supply evidence that the limits of tolerance of the patient have been overstepped and that medication must be reduced

Another less distasteful form of medication is the administration of ammonium chloride which is probably transformed into hydrochloric acid and urea in the body, and so produces acidosis (183) This can be readily taken in doses of 1 gram given in a glassful of water, ten and sometimes twelve times a day Throughout this treatment, diet should contain little calcium Although not easy to administer, this medication is far more effective than other more simple forms and in eight of our cases has increased the average excretion of lead three-fold Ammonium chloride is now employed instead of phosphoric acid because it is less distasteful

Sodium bicarbonate likewise is effective If given in large doses (20 to 40 grams a day) it more than doubles the elimination of lead and therefore produces an effect approximately similar to that of potassium iodide The suggested explanations for its action are given in the chapter on Excretion It is chiefly valuable when nephritis prevents the use of acids or potassium iodide, but even it must be employed guardedly

Our observations, particularly on animals, indicate that there is no use in attempting to continue treatment until elimination of lead is complete As this would doubtless require several years, it is probably desirable to eliminate only the most easily mobilized lead Our very prolonged observations suggest that after a certain point elimination of lead becomes progressively more difficult When this stage is reached, it seems more practical to favor retention in the bones by maintenance of a positive calcium balance

Of the other recognized methods of dealing with lead poisoning,

the only one which seems to yield noteworthy results is catharsis Although this has been used successfully since the Middle Ages, the exact means by which it brings relief had not been determined, so far as we could ascertain, until experiments were performed in this laboratory to determine its effect This treatment does not increase the rate of excretion of lead markedly, it merely improves the general condition of the patient by cleansing the bowels and preventing the constipation which is characteristic of lead poisoning Thus it cannot be considered a "specific," but its use is quite justified by the beneficial results which it produces Saline cathartics are apparently the most satisfactory For continuous use, Carlsbad salts are best, but magnesium sulphate is effective and may be employed Regulation of the bowels without drugs is, of course, a much better type of treatment than periodic purging

The value of bipolar electric currents in the prevention and treatment of lead poisoning is disputed Oliver (336) used this method on the theory that in passing through the body electric currents split the salts of lead into ionic constituents which migrate toward the different poles—the acid radicals to the positive, the basic to the negative electrode Lead should consequently be deposited at the negative pole In 1913 Oliver reported that such currents cause marked improvement of symptoms and that lead really could be found in the water of the electrodes In most instances, when the urine had contained lead before electrical treatment, "less lead was found in the urine after the bath" (336) Since this work was published, several clinical reports have appeared which suggest that good psychical results follow the use of electricity in plumbism, but no further experiments have been performed which give support to the treatment Goadby, Oxley, Schnitter and Bottrich are all opposed to this form of medication Goadby (167) found that after lead has been ground up with liver tissue, electric currents are unable to remove it, and that they are quite ineffectual when applied to "leaded" animals Oxley (319) believed that the current must necessarily be too low to remove more than a negligible quantity of lead, and that a very large proportion would be carried by other ions In a series of cases he could observe no improvement in the symptoms after electrical treatment, and could detect no lead in the water

about the electrodes Analysis showed that lead was not present in the urine of a case of chronic plumbism either before or after use of the electric current Experiments on two rabbits confirmed these clinical observations In Schnitter's (401) experience with twelve patients, similar treatment produced no visible effect upon the lead line, the degree of anemia, the amount of stippling in the blood, or the general condition. Because the work of these three investigators seems so convincing, we have made no attempt to further establish the value of electrical treatment This would necessitate analysis of the feces as well as of the urine and electrolyte solutions for any lead which might be excreted. Since lead exists in the body for the most part as insoluble phosphate in the bones or as un-ionized phosphate in the blood stream, the probability that it can be affected by electricity is small.

Quite recently Ockerblad (334) has reported that hyposulphite of soda (0 6 gram injected into the vein daily) is beneficial His use of this drug in a case of acute lead poisoning was suggested by the work of Ravaut (378) who administered it in cases of arsphenamine dermatitis to produce an insoluble sulphite of arsenic. McBride and Dennie (286), who have employed it in the treatment of both arsenic and mercury poisoning, also point out that sodium hyposulphite may be useful in such other acute metal intoxications as plumbism Their treatment is based upon the theory that when the metal encounters this salt in the body a sulphite is precipitated which is insoluble and therefore not dangerous to the organism That the small quantities of ionized lead in the body fluids may be temporarily reduced in this way, seems quite possible, but only when considerable thiosulphate remains in the blood stream. Although this method of treatment does not prevent the entrance of additional lead into the blood or a re-transformation of the insoluble lead into a toxic salt, it may prove valuable in dealing with acute active lead poisoning and deserves further study

Special treatment of toxic episodes. *Colic* Treatment often brings rapid relief to the severe pain of typical lead colic, even when it consists only of such general measures as the application of local pressure, heat, and moisture on the abdomen. Enemas, although they are sometimes difficult to administer because of rectal spasm,

and ineffective because of rapid expulsion, at times prove helpful in stopping pain If diagnosis is certain and all possibility of an acute surgical abdomen is ruled out, catharsis is the best treatment In our experience, magnesium sulphate (given in doses of one ounce) has proved most satisfactory If this is not effective, the dose may be repeated Adequate doses of such drugs as atropine, nitroglycerine, or amyl nitrite should also be used, but resort should be had to opiates, morphine and the like, only under extreme conditions

In five successive cases of colic, the intravenous use of calcium chloride (15 cc of a 5% solution given very slowly) promptly relieved the pain,—but this observation requires corroboration With our patients, benzyl benzoate has seemed ineffective Until the acute manifestations have subsided, a light diet containing much milk and calcium lactate (2 grams daily) should be given to favor storage of lead in the bones, but as soon as the crisis has passed efforts should be made to favor elimination of lead from the body

Palsy During the development of palsy, treatment which favors storage of lead should be used, and elimination should not be stimulated until the neuro muscular signs become stationary Local treatment by galvanism, massage and splints is then desirable, and the use of strychnine is frequently said to be effective This treatment should be continued for many months inasmuch as improvement nearly always results in the end

Encephalopathy Although the usual treatment for mania or convulsions is of course necessary, the prompt storage of lead is obviously essential in the treatment of encephalopathy But this manifestation is so rarely seen now, that the effectiveness of treatment has not yet been determined Of the six positive cases we have seen, three died without such treatment The three patients, who were given large quantities of milk and calcium lactate with a little sodium bicarbonate, recovered promptly A seventh somewhat questionable case, that of a child who had lead in the excreta, also recovered when milk was administered in large quantities McCord (289) has recently reported a case at the Cincinnati Academy of Medicine of prompt recovery from an acute lead mania following the administration of alcohol When the acute symptoms have subsided, measures to increase the elimination of lead seem advisable

After any of these acute manifestations of plumbism, return to a lead hazard is distinctly undesirable.

Late manifestations. The treatment of arteriosclerosis and of the arteriosclerotic nephritis which develop after long exposure to lead is similar to that for the more usual forms of these manifestations It sometimes appears desirable to increase the rate of excretion of lead even in these cases but this should be done only with caution, as patients with severe nephritis often cannot well tolerate inorganic salts, particularly potassium iodide and acids It is necessary to administer these only with great care Sodium bicarbonate is usually the safest drug to use

Summary. The treatment of lead poisoning, therefore, resolves itself into treatment for the toxic episodes and mobilization of the stored lead. Various treatments favoring elimination of stored lead are discussed, among them several new methods During severe intoxication it is apparently wiser to facilitate *storage* of lead by ingestion of ample calcium, rather than to set it free After the acute toxic episodes have passed, *elimination* of lead may be accelerated by a low calcium intake, acids and their ammonium salts, sodium bicarbonate, and potassium iodide Caution should be exercised in using these forms of treatment on patients with nephritis

XX THE PREVALENCE OF INDUSTRIAL LEAD POISONING IN THE UNITED STATES

ALICE HAMILTON

The literature of industrial lead poisoning in the United States dates probably from the publication by Samuel Dana of Lowell in 1850 of a translation of "A Treatise on Lead Diseases" from the French of Tanquerel des Planches A second impetus to the study of this subject was given in 1883 by J J Putnam (370) of Boston, who by detecting lead in the urine showed how much more prevalent was this form of intoxication than had been supposed The material both clinical and pathological, from American sources, however, remained very scanty till the beginning of this century During the last ten years very valuable additions have been made to our knowledge not only of the action of lead on the human body but of the prevalence of

occupational plumbism in various trades, the period of exposure before poisoning occurs, the different forms which the intoxication assumes under different working conditions, and the dangers which must be guarded against if men and women are to be protected when engaged in lead work The following is a very brief summary of what has been published in this field in the United States

A word must be said first as to the sources of our information on the subject There are many difficulties in the way of the searcher for accurate data as to the incidence of plumbism in any one occupation for we have in this country no system of sickness insurance, such as obtains in the older industrial countries and provides an invaluable body of statistics, nor is there any uniformity in our factory inspection records, each state having its own methods and standards The United States Census Reports cover deaths only and so do the records of the Industrial Life Insurance Companies It follows that though it is sometimes possible to obtain full and accurate records from individual plants it has never yet been possible to say how much lead poisoning has occurred in any given industry in any given period of time,[5] and the statements made in this paper are only approximately accurate, for they are based upon incomplete investigations

In lead mining the United States is under a great disadvantage as compared with the older countries because while in European lead mines plumbism is almost unknown[6] because old mines are deep and yield only the very poorly soluble lead sulphide ore, the mines in our western states are still near enough the surface to yield oxidized ores, carbonate, sulphate and oxides which are far more toxic Missouri mines are now down below the water line but in Utah, especially in those areas where the mines are dry and dusty there is an amazing amount of plumbism Information secured by Arthur Murray (324) for the United States Bureau of Mines showed that in Utah alone there were 468 cases of plumbism in lead miners in 1919 and 1920, the rate being 6 7 per 100 employed in 1919 and 5 0 in 1920, while in the

[5] The most nearly complete examination in this field was that made by the United States Public Health Service, of lead poisoning in the pottery trade in New Jersey, Pennsylvania, Ohio and West Virginia which was published in 1921 as Bulletin No 116

[6] According to Oliver (Dangerous Trades, London 1902) no lead miner has died of plumbism in England for many years, and according to Rambousek (374) it was practically unknown among the miners of galena ore (lead sulphid) in Bohemia However, Biondi (374) asserted that he had seen lead poisoning among the galena miners of Sardinia

shallow, dusty mines of Frisco the rate for 1920 was no less than
100 employed

In smelting and refining lead there is a high rate of lead poiso
this industry is very important in the United States and gives
ment to a large force of men. No recent investigation has be
in this country and the only figures available are those collecte
Bureau of Labor Statistics in 1913 (471) During that year th
at least 1770 cases in 19 plants employing some 7500 men an
these were 41 encephalopathies, 35 cases of paralysis and 16
The British rate for 1912 was only 1 8 per 100 Intervie
167 smelter men, suffering from plumbism, showed that the
had become intoxicated after only a short exposure. Eight
worked less than a month, 121 were exposed less than six
132 less than a year, and only 35 had worked more than a
whom 5 had worked more than five years

That lead poisoning is caused chiefly by the presence of finely
lead compounds in the air is abundantly shown in the smelting and
industry. Thus, three smelters in which ore hearths (or Scotch
with their enormous flue and bag-house attachments, are used were
ing 13 5 per cent of the total laborers in the trade but they had
cent of the cases of plumbism, 54 3 per cent of the cases of palsy,
cent of the fatal cases and 70.7 per cent of the encephalopathi
greatest danger lies in the fumes of lead oxide and sulphate from
naces and next to that in the fine dust from the flues and bag-hou
two large smelters which have been recently investigated have
many improvements and there is certainly less danger now in th
of lead work than there was ten years ago, although it is probable
refining end of the industry has undergone less improvement
smelting because all over our country there are cheaply constru
cheaply managed refineries, practically junk shops, where no effor
to control dust or carry off fumes

Zinc smelting may involve similar dangers if the blende
much lead, as it does in Colorado Missouri blende conta
0.5 to 1 per cent, and New Jersey blende is almost lead-free

[7] Of the 19 plants included in this study, only two had full medical records
four no information could be secured Therefore, the number of cases whic
occurred in 1912 was greater than 1769, the number of records actually secured

Metallic lead is used in a wide variety of industries where it is cast or molded into various shapes Lead wire, sheet lead, pipe, machine parts, plumbers' goods, bullets and shrapnel, picture frames, coffin ornaments, grids for storage battery plates, car and can seals, stoppers for bottles and for basins, lead foil, printer's type, all are objects the making of which involves the handling of solid or molten metallic lead, and cases of industrial lead poisoning are on record in connection with all of these occupations Other industries involving an extensive use of metallic lead are the making and use of solder, lead burning with an oxyhydrogen flame, and lead tempering of machine parts, piano wires, magnetos, etc The plumbers' trade is less a lead industry than formerly, but repair work with "joint wiping" means exposure to lead Among 560 cases of industrial plumbism collected by the Illinois Occupational Disease Commission, 19 were in plumbers

The danger of plumbism in all these trades is comparatively slight and easily controlled It is only necessary to carry off the fumes from lead pots, which is usually a simple matter, and to insist on strict cleanliness, no scattering of lead scrap and dust But the very lack of evident and immediate danger in the use of metallic lead seems to induce a neglect of ordinary precautions and in many plants where soldering or re melting used metal or casting is carried on, there may be more lead poisoning than in a very well managed white lead plant Lead burners, who melt together two surfaces of lead with an oxyhydrogen flame, have probably the most dangerous work among the metallic lead occupations because of the great heat used Next to that would come tempering metallic objects in a bath of lead, and later on brushing off the gray oxide coating which has formed

Brass foundry work, especially pouring, is a not uncommon source of lead poisoning, for brass usually contains lead, sometimes more than 10 per cent, and both founders and polishers may suffer from typical plumbism

The printers' trade is the most important of the metallic lead trades, and yet the exposure to lead is very slight Typical lead poisoning has never been common in this industry,[5] but the trade is a notoriously

[5] In 1916 a group of 100 Chicago printers were examined by John D Ellis, and a similar group of Boston printers by Walter W Palmer They found 18 men suffering from symptoms suggestive of chronic plumbism and with a history pointing to the same This made a rate of 9 per hundred

unhealthful one in every country (474) Recent statistics gathered by the International Typographical Union (New York Local) show that certain chronic diseases not clearly connected with lead are very prevalent among printers There is an excess of early deaths from heart disease, chronic disease of the kidneys, apoplexy and paralysis. Thus, among white males in the registration area, only 18 6 per cent of the deaths from heart disease occurred in the age groups under forty-five years, but among the printers 26 per cent was the figure The deaths from apoplexy and paralysis show an even greater contrast, only 9 1 per cent of the deaths from these causes occurring under forty-five years in the general group but 20 4 per cent among printers.

Tuberculosis is, however, the occupational disease of the printer and apparently it is connected with this exposure to lead, for in every country it has been found that when lead fumes and dust in the printing shops were controlled the tuberculosis rate always fell.

The statistics of the Metropolitan Life Insurance Company for 1917 show that printers have a higher tuberculosis rate and die from this disease at an earlier age than workers in non-metallic lead, such as painters Tuberculosis is the cause of 34 1 per cent of the deaths of printers, of only 21 9 per cent of the deaths of painters and varnishers, and the former average only 33½ years at death, the latter almost 40 years A possible explanation for what is apparently the effect of tiny quantities of metallic lead dust inhaled over a long period of years, is to be found in the paper by Fine (137) to which the reader is referred

Lead is roasted to form the oxides Litharge, red lead, and orange mineral, for which there is a very extensive demand in industry The danger comes from fumes from the furnaces, very much lessened in late years by the introduction of mechanical devices, and from dust in transporting the oxides, dumping, grinding, screening, and packing The dust problem is much harder to solve than fume control The newer methods of roasting require fewer men, but the number of oxide men nevertheless is decidedly on the increase because of the constantly growing demand for oxides to make storage batteries, enamels for sanitary ware, glaze for tiles and terra-cotta, glass and varnish, paint and rubber, to mention only the most important uses. Lead poisoning in oxide men is likely to be of rapid development and fairly severe (472).

The making of storage batteries involves the use of great quantities of lead oxides and this is not only one of the most extensive of the dangerous lead trades but the one that is increasing most rapidly In Great Britain it is at present the greatest single source of plumbism The rate in the five large plants in the United States in 1914 was a little less than 18 per 100, but this figure was below the truth, for almost no records could be obtained from the worst of these plants The men engaged in casting the lead grids had a rate of only 1 7 per cent, but those employed in mixing the oxide paste had a rate of 40 per cent, and the pasters a rate of 19 4

Enamels for bath tubs, sinks, and other plumbing goods also require a great deal of red lead, and here also the number of men exposed is continually growing for we make more new bath tubs every year These enamels contained in 1911 from 1 5 to 20 per cent of soluble lead (soluble in human gastric juice) Lead renders the enamel more fusible and flexible The dry enamel is scattered over the ware while it is red hot and there is no way of carrying off the lead laden dust so as to prevent contamination of the air, while the heat and exertion are too great to allow the enameller to wear a respirator However, efforts to lessen the dust have been made of late and it is highly probable that plumbism is not so prevalent in this industry as it was in 1911 when 54 out of 148 men, who were examined, had unmistakable signs of chronic lead poisoning (469)

The manufacture of rubber, especially footwear, mechanical rubber, and automobile tires, uses now less lead relatively but more absolutely because so much more rubber is manufactured each year The lead is usually litharge, sometimes sublimed lead sulphate, and it is added to crude rubber to accelerate vulcanization and to make the product heavier Lately organic accelerators have been taking the place of lead and yet so great is the increase of rubber manufacture that there is more lead sold now to rubber factories than before Only a small proportion of the employees come in contact with lead but unless great precautions against dust are adopted there may be a high rate of plumbism among the compounders and mixers In one factory there were only 25 out of 1200 employees who handled lead but 11 cases of plumbism occurred in one year (473)

White lead is made by two methods— the old Dutch process and the

Carter. The former is slow and requires much hand work, the latter is dusty but quick and largely mechanical. This industry has undergone very radical improvement in the last ten years and almost all white lead works are now equipped with excellent mechanical devices to prevent dust, with ample washing facilities, a clean lunch room and washable working clothes, provided and laundered by the management There is always a physician in charge of such plants Cases of acute lead poisoning are fairly infrequent at present but every now and then a serious case of acute and rapidly developing lead poisoning does occur, showing that susceptible men run a risk in such work Negroes are universally found to be more susceptible, especially to the cerebral forms of plumbism

The painters' trade in every country has been brought under less complete control than any other lead trade, and it is the one that shows the least improvement in the rate of lead poisoning For this reason the French are insisting on the abolition of lead paint and they have already closed their white lead factories and forbidden the use of lead paint on buildings On the other hand, the British (338) (12) and German (255) (404) authorities on industrial hygiene lay stress on the toxic constituents other than lead which are present in almost all paint and also in paint removers, shellac and varnish These are volatile fluids such as turpentine, benzene, petroleum distillates and wood alcohol, all of which are capable of producing more or less serious intoxication and, according to this view, are really responsible for much of the ill health of painters which is usually attributed to lead (See abstract of controversy before the International Labor Bureau of the League of Nations, by E L Collis in the *Journal of Industrial Hygiene*, 1923, 5, 52 [9])

Nevertheless, great efforts have been made in Great Britain and in most Continental countries to minimize the dangers of lead paint by prohibiting dry rubbing down when lead paint is used, prohibiting the mixing of dry white lead by the painter, restricting the use of white lead paint in interior work, etc Fortunately the American tendency

[9] The Draft Convention of the International Labour Office of the League of Nations which was formulated at the October 1921 session, and submitted to nations which are members of the League, prohibits the use of white lead or sublimed sulphate of lead in the interior painting of buildings

to speed up the job leads more and more to the abandonment of dry rubbing down in interior decoration, except on very high class work, but it is still done in the painting of Ford automobile bodies and of practically all vehicle wheels

The general impression obtains that less lead paint is used now than formerly, but interviews with white lead producers do not confirm this There has certainly been an increased production of white lead in the United States since the War, and the painting trade is still the largest consumer, with the potteries coming next A new danger to the painter has appeared recently in the introduction of the "spray gun" which has met with an enthusiastic reception and has spread rapidly It is now used for painting all sorts of factory goods, not only small objects which may be placed in a cabinet with an exhaust, but automobiles, wagons, railway and trolley cars, agricultural machinery, furniture, and even the interiors of buildings It is easy to see how this increases the danger of lead poisoning, for a spray of finely divided droplets, each carrying its tiny load of lead, is hard to control The experiments of Sharpe (418), of Toronto, have shown that in such work the lead content of the air may go far beyond the limit of safety, and that the ordinary respirator lets a dangerous amount pass through The Bureau of Mines has recently shown that a gas mask can be constructed which will hold back the lead, but it is certainly doubtful whether painters will consent to work in such a mask

The painters' trade is still at the head of the list of occupations which give rise to plumbism, as is indicated by many recent reports in the United States (468) (470) (201) It must be remembered that an allied industry, that of the glaziers, is also attended with the use of white lead This is a constituent of glaziers' putty, and recently four glaziers, working in a New York shop, were found to be excreting lead in the urine

Pottery is the next most important of the white lead using industries and one that is increasing steadily in extent in the United States. Lead glaze is used on all so called white ware (table and toilet ware), on smaller sanitary ware (not earthenware bath tubs), on art ware, yellow ware and Rockingham, and on glazed tiles used for interior decoration and occasionally on glazed roof tiles White lead is the

form most used, next to that comes red lead In some potteries the lead is "fritted," that is, it is added to the raw constituents of the glaze and fused with them and thus changed in part to the insoluble lead disilicate, a procedure which greatly lessens the danger, but which is unfortunately not often used in the United States, the lead being commonly added after fritting is over The study made recently by the United States Public Health Service (Bulletin 116) shows a very high rate of plumbism among the men and women who glaze and decorate pottery and tiles The physicians examined 1809 men and women potters and found a rate of at least 13 5 per hundred employed, while the rate in British potteries in 1913 was only 0 9 per hundred, one-fifteenth of the American rate

The prevention of lead poisoning may be difficult of execution, but the principles which must guide it are very simple In almost all cases of industrial plumbism and in all serious cases, it will be found that the workman has been poisoned by lead which entered his body with the inspired air It is unnecessary to enlarge on this point, for the preceding chapters provide the explanation for a fact which has been observed repeatedly in practice since it was first announced by Tanquerel des Planches a hundred years ago He said, "All the characteristic traits of the primary effects of plumbism may be quickly observed in workmen who are habitually in an atmosphere of lead dust and vapors None of the primary effects are found among workmen who handle lead in a fixed state"

The practical experience of British and German factory inspectors bears out abundantly the assertion that the air must be kept free from lead dust and lead fumes (fume is a suspension of extremely fine particles of lead oxides and sulphate) if serious poisoning is to be prevented Of secondary importance is the provision for personal cleanliness, but of course this should never be neglected

Since the susceptibility to lead varies enormously in different individuals, and since it is impossible to detect this susceptibility at the time when the worker is employed, it is essential to have a medical examination of all lead workers at regular intervals If any case of lead poisoning is discovered, the worker should at once be suspended from lead work and given other employment, preferably out of doors, till all symptoms of lead poisoning have disappeared

XXI ADDENDUM

Lead in liquor

In a few cases in which there had been no industrial exposure, lead poisoning has been caused by the consumption of alcoholic drinks containing lead These beverages were illegally made—presumably with ordinary household appliances Because lead was found in a few samples, various liquors known to be used commonly by saturnines have been systematically investigated The results are grouped in the following table

The occurrence of lead in liquor

	mgm of Pb per liter of liquor
Distilled liquor	5 15
Distilled liquor	1 41
Distilled liquor	52 70
Distilled liquor	18 30
Distilled liquor	5 84
Distilled liquor	6 25
Distilled liquor	0 60
Wine	74 25
Wine	0 00
Wine	75 40
Wine	1 38
Wine	1 62
Wine	1 09
Wine	0 40
Wine	0 00
Wine	0 26
Wine	1 04
Wine	trace

It is interesting and significant that of eighteen samples of liquor analyzed, two only were negative In the preparation of at least one kind of distilled liquor a lead worm had been used as a condenser That wines should contain lead is not surprising as fruit acids (chiefly tartaric) are an excellent solvent for most lead compounds and can easily dissolve it from crocks glazed with a lead glaze metal vessels containing solder, or from copper vessels tinned with an inferior tin-lead alloy Consequently, contaminated liquors constitute a definite source for so called "normal" lead

The literature of plumbism contains many references to poisoning from liquors purposely or accidently contaminated with lead Chevallier and Olivier (79) cite an instance of an epidemic of lead poisoning caused by the use of litharge in treating "green" wine Campbell (62) and Allden (6) both report cases of lead poisoning occasioned by home made wine which had dissolved lead from the containers Bidwell (37) reports contamination of liquor with lead and recently mention has been made (500) of several cases of lead poisoning in England caused by lead from the vitreous enamel coating of the cast iron tanks used in the manufacture of beer Vaughan (475) also cites instances of lead poisoning after the consumption of "moonshine" whiskey which had evidently been contaminated by the stills.

The wide-spread use of containers glazed with lead compounds and the careless use of leaden or soldered containers in the preparation of home-made beverages is therefore a matter of concern from the view point of health

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ETIOLOGY OF SCARLET FEVER

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Scarlet fever is in all probability a very old disease The regions in which the malady originally arose are a matter of uncertainty There are some who believe that the Plague at Athens was a malignant form of scarlet fever, an interesting assumption in view of the present relatively low case fatality and the ominous variability in severity of outbreaks in the past Fairly accurate descriptive records of scarlet fever appear in the literature as early as the middle of the sixteenth century and recur with increasing frequency and definiteness up to the time of Sydenham For many years the disease was confused with measles, erysipelas, diphtheria and certain septic processes Sydenham, who first employed the name scarlet fever clearly differentiated it from measles by his careful description of the disease as it appeared in London from 1661 to 1675, and laid the foundation of an accurate knowledge of its special characters In spite of this valuable contribution the existing confusion did not disappear, and many physicians still confounded it with diphtheria and certain septic anginas With the increasing volume of medical literature and better facilities for the communication of ideas, scarlet fever became more and more clearly defined as a clinical entity Confusion, however, with diphtheria frequently occurred, even, down to the times of accurate diagnosis by means of bacteriological methods In fact even today the inability to differentiate scarlet fever from certain septic conditions of the throat, associated with erythematous rashes, continues to plague the mind of the diagnostician In spite of these diagnostic difficulties clinical differentiation of scarlet fever from other exanthemata has been possible for a long enough period of time to determine clearly its contagious nature and to permit illuminating epidemiological and clinical studies

The contagious element in scarlet fever is probably always derived

from a previous case In most instances it is taken directly into the mouth or nasopharynx by the inhalation of air charged with minute droplets of saliva or mucous projected from the mouth or nose of the infected individual Other important sources of contagion are the purulent discharges from infected paranasal sinuses, from suppurative inflammation of the middle ear and lymph glands, secondary conditions that constitute the most frequent and distressing complications of the disease There is much evidence to support the view that the causative organism survives in the dry state in a virulent form for long periods of time Contamination, therefore, of clothing or personal articles of any kind with infective matter may serve as a means of conveying scarlet fever Formerly the belief was quite prevalent that flakes of skin given off during the period of desquamation were the most important vehicle of the contagion, and quarantine regulations were roughly founded on time periods corresponding with the duration of the desquamative stage Current opinion holds that the contagious element is not present in the skin in the late stages of scarlet fever, a somewhat curious fact, inasmuch as the rash is the most distinctive clinical manifestation of the disease The rôle of the healthy carrier in spreading scarlatina is undoubtedly of great importance but determined accurately as yet in only a very few instances because of the uncertainty concerning the etiological agent That such types of carriers do exist there can be no doubt, and Bliss (1) has been able to trace a small epidemic of scarlet fever to such a source Another interesting means of the wide dissemination of scarlet fever is an infected milk supply and numerous undoubted outbreaks have arisen from the consumption of contaminated milk The clinical and epidemiological evidence, therefore, that has been collected indicates that the causative agent of scarlet fever is present in the throat secretions and the discharges from suppurative foci in patients throughout the illness, and for a considerable period of time during convalescence It resists exposure to light, and in the dry state may retain its infectivity for many months Healthy carriers and atypical attacks of the disease are not an infrequent occurrence In all probability the udder of the cow may become infected with the specific virus, and the milk obtained from this animal may serve as a vehicle of infection

Notwithstanding these excellent clinical and epidemiological studies which have ensured the easy recognition of typical attacks of the disease, and which have furnished the essential data for useful quarantine regulations, the causative organism of scarlet fever has remained unknown Experimental studies have been publishd from time to time, suggesting that the infective agent belongs to one or other of the principal groups of microorganisms, such as the pathogenic bacteria, protozoa, and the so-called ultramicroscopic viruses As a bacterial cause, Streptococcus hemolyticus has aroused much interest and stimulated more or less continuous investigation because of its constant association both with the uncomplicated and complicated forms of the disease Certain observers have discovered inclusion bodies in leucocytes and in epidermal cells which they have thought indicative of a protozoan cause for scarlet fever Finally, scientific opinion seized upon those mysterious living bodies commonly designated as filterable viruses as the most probable cause of the disease This latter view has become most widely accepted and is the usual etiology assigned in text books in spite of the fact that no real evidence has ever been produced to show that any such microorganism exists either in the throat secretions, tissues or blood of an individual suffering from scarlatina

Both Mallory (2) and Dohle (3) have made the suggestion that scarlet fever may be due to a protozoan infection In 1904 Mallory observed in four cases of scarlet fever certain bodies whose varying morphology strongly suggested that they might have been stages in the developmental cycle of a protozoan They occurred in and between the epithelial cells of the epidermis and free in the superficial lymph vessels and spaces of the corium They formed a series of bodies including definite rosettes, which closely resembled those seen in the asexual development of the malarial parasite There were also certain coarsely reticulated forms which he thought might represent stages in sporogony Mallory was of the opinion personally that these bodies were protozoa and bore an etiological relationship to scarlet fever but he did not regard their significance as established Confirmatory observations were subsequently made by Duval (4), Bernhardt (5) and v Provazek (6) Similar bodies, however, were later found by Field (7) in other conditions and they

finally came to be looked upon as peculiar products of cell degeneration and not as living forms with a specific relationship to scarlet fever

In 1912 Dohlé, on examining the blood smears from thirty cases of scarlet fever, found within the cytoplasm of the neutrophilic polynuclear leucocytes multiform inclusion bodies These inclusions were present in a large percentage of all leucocytes and by special methods of staining revealed themselves as intermediate in intensity between nucleus and cytoplasm. In a later communication certain of these inclusions are designated as "Spirochaeta scarlatinae," and are assigned both diagnostic and prognostic importance Although numerous observers confirmed Dohlé's observations on the presence of leucocytic inclusion bodies, further study revealed the fact that they are present in practically all febrile conditions, in chronic pyogenic infections without fever, in certain severe injuries, and occasionally in normal human beings In all likelihood they result from nuclear degeneration not infrequently observed in septic states and have no specific bearing on the etiology of scarlatina The evidence offered in favor of the protozoan origin of scarlet fever has never stood the test of close scrutiny

The belief that scarlet fever is due to an unknown virus, probably of filtrable character, is based largely upon the results of attempts to communicate the disease experimentally to animals A number of observers have reported scarlatina-like manifestations in monkeys inoculated with infective material from active human cases of the disease Among these observations the most interesting are those of Levaditi, Landsteiner and Prasek (8), who, by the inoculation of anthropoid apes, seem to have produced what in all likelihood was true scarlatina Exudate from the throats of individuals with scarlet fever was rubbed into the tonsils of apes and defibrinated blood injected subcutaneously, and in one instance material from a suppurating lymph gland The animals, after an incubation period of about three days, are described as having a typical angina with characteristic exudate, enlargement of the follicles of the tongue, a generalized exanthem resembling that of scarlet fever, and in certain instances when the animals recovered desquamation of the skin. There was also present the characteristic lymphoid hyper-

plasia, and the histological lesions in the skin resembled those seen in scarlet fever In all the animals presenting such a picture S hemolyticus was present, either in the blood or in the local lesions in the throat Levaditi, Landsteiner and Prasek, however, did not think that streptococcus was accountable for the manifestations, inasmuch as when pure cultures of this organism were obtained from the infected animals or from human beings and inoculated into fresh apes, the phenomenon described could not be reproduced They do not state that the organism of scarlet fever is a filtrable virus, but simply say that it is of unknown characteristics Cantacuzene (9) and Bernhardt (10) claim to have induced a similar series of phenomena by the inoculation of monkeys of a lower order with human material Levaditi, Landsteiner and Prasek failed to produce in a large series of lower monkeys the disease syndrome manifested by the apes, nor were subsequent investigators more successful in confirming the onservations of Cantacuzene and of Bernhardt From the failure to discover an organism of known characteristics, rather than from any positive evidence has grown the belief so generally held that the etiological agent of scarlatina is an ultramicroscopic virus

During the many years that investigators have searched for the causative agent of scarlet fever, and with the varying emphasis attached to one or another species of parasite from time to time, the constant relationship to this disease of one organism S hemolyticus has become more and more significant. As early as 1885 Crook (11) reported the presence of streptococcus in the blood and organs of individuals dying of scarlet fever Loeffler (12), in addition, found this organism to be present in certain types of necrotic angina associated with scarlet fever and was furthermore successful in isolating the germ in pure culture At this time Klein (13) likewise isolated a streptococcus from the tissues of patients with scarlatina, which he named Streptococcus scarlatinae In 1885 the latter observer while investigating an outbreak of fever among certain cows belonging to a farm at Hendon, England, isolated from ulcerative lesions of the udders and from certain viscera, a streptococcus which he considered to be identical with Streptococcus scarlatinae This observation was not only of great interest but also of very great impor-

tance because the milk obtained from the infected cows was shown to have been consumed by persons who subsequently developed scarlet fever These early observations of the frequent relationship of streptococcus to scarlatina were soon confirmed by many students of the disease in different parts of the world In 1900 Baginsky and Sommerfeld (14), reported the constant presence of streptococcus in the throat during the characteristic angina in seven hundred cases of scarlet fever They also found this organism frequently in the blood, bone marrow and internal organs of patients dying of this disease Other observers found streptococcus in the blood of fatal cases of scarlet fever in as many as 70 per cent of the individuals studied Hektoen (15), furthermore, found the organism in blood in 12 per cent of patients during life, and his observations are of especial interest in that they indicate that the usual bad prognostic import of this phenomenon does not necessarily hold for scarlet fever

In addition to the presence of streptococcus in the throat and blood of individuals with scarlet fever, this organism has also been proven to be the most frequent cause of the septic complications of the disease Many times in septic foci streptococcus has been found in pure culture, and it is an old observation that convalescent individuals with discharging suppurative lesions are especially likely to give rise to return cases of scarlet fever showing that in such lesions the causative virus persists in an active form for long periods of time

Such widespread and constant association of S hemolyticus with scarlet fever has led some investigators to propose the view that streptococcus is the etiological agent of this disease Certain observers, on the other hand, oppose this belief and have considered it more likely that streptococcus plays in scarlatina the rôle of a secondary invader. The objections of this latter group to the etiological significance of streptococcus are based upon certain important considerations As is well known streptococcus is an organism of very widespread distribution and gives rise to a variety of pathological lesions such as abscess formation, cellulitis, septicemia and numerous other conditions Frequently the same individual may have throughout life repeated streptococcus infections, one attack not seeming to confer immunity against subsequent invasion of the tissues by the same organism The latter condition of affairs is especially true of

erysipelas, one of the most characteristic of the streptococcus diseases On the other hand, scarlet fever, in sharp contrast to other streptococcus infections, is a fairly definite clinical entity and one attack appears to give rise to an immunity of life long duration This peculiarity of scarlet fever might have been explained had it been possible to prove that the streptococcus associated with scarlet fever differed specifically from the hemolytic streptococci causing the various septic processes However early cultural and biochemical studies have failed to demonstrate any significant differential characteristics by means of which Streptococcus scarlatinae could be separated biologically from similar streptococci found in other diseases When grown in fluid or in solid media, hemolytic streptococci resemble one another very closely, whatever be their source It is true that certain constant differences can be brought out by means of fermentation of various carbohydrates, but such variations as exist apparently do not bear any specific relationship to a single disease process, and have been of but little aid in determining the etiological significance of streptococcus in scarlet fever In addition to this objection Jochmann (16) has emphasized especially his failure to find streptococcus in either the blood or tissues of individuals dying in a few days from malignant forms of the disease Since, therefore, types of streptococci indistinguishable from those seen in scarlatina are found in a great variety of disease conditions, and since the quality of the immunity in this disease differs widely in its duration from that observed in other streptococcus infections, and finally because of Jochmann's contention that streptococcus is not present in certain malignant types of scarlet fever, the conclusion has been drawn that streptococcus cannot be the cause of the disease

An effort to meet these objections has been made by the group of investigators who believe that streptococcus is the etiological agent of scarlet fever The observation by Baginsky and Sommerfeld of the constant presence of S hemolyticus in the throats of all cases of scarlatina, an observation later confirmed by others has done much to offset the inferences drawn from Jochmann's failure to find it in a few instances of fulminant types, especially since we now know that the organism in the latter cases may have been localized in some inaccessible area Attempts were made in addition to explain the

immunity in scarlet fever and to establish the type specificity of the scarlatinal streptococcus Moser (17) and Moser and Pirquet (18) have claimed that scarlatinal convalescent serum agglutinates to a higher titer Streptococcus scarlatinae than does control serum from other diseases Furthermore, they have prepared polyvalent serum from horses, using the streptococcus of scarlet fever as antigen and have studied the capacity of such sera to agglutinate specifically various strains of scarlatinal streptococci The latter strains were agglutinated in dilutions of 1·1000 or over, whereas hemolytic streptococci from other sources were not specifically agglutinated As a consequence of these observations Moser and Pirquet believed that the streptococcus of scarlet fever differs specifically from apparently similar strains isolated from instances of erysipelas, phlegmon and puerperal sepsis Meyer (19) and Rossiwall and Schick (20) have confirmed the results of Moser and Pirquet Unfortunately, however, certain later studies by Hasenknopf and Salge (21), Aronson (22), and Neufeld (23) failed to support the earlier ones and grave doubt was thrown upon the specificity of Streptococcus scarlatinae

Other interesting facts which indicate the specific relationship of streptococcus to scarlet fever have come from the studies of Gabritchewsky (24) on the specific prophylaxis of scarlet fever by means of a vaccine prepared from S scarlatinae, and of Moser (25) on the therapeusis of the disease by means of a specific antistreptococcus serum Gabritchewsky and his coworkers immunized a large number of individuals against scarlet fever with a vaccine prepared from hemolytic streptococci isolated from scarlatina During the process of immunization certain phenomena occurred which were highly suggestive of the clinical manifestations of scarlet fever In the majority of instances an area of erythema and swelling averaging 15 cm in diameter developed at the site of the vaccine injection appearing in from eight to twenty-four hours and lasting about forty-eight hours In general the erythema was diminished or absent following a second injection some ten days later In about 15 per cent of the individuals inoculated a general reaction was observed This general reaction consisted in fever of 1°C or so, leucocytosis and an erythematous rash, having the characteristic distribution of the exanthem in scarlet fever Some of those inoculated showed the

typical angina and strawberry tongue peculiar to the disease and in a few instances signs of renal irritation were observed In general individuals who were recovering from the disease or who had had it some years before failed to show either a local or general reaction Administration of Moser's antiscarlatinal serum before the inoculation was shown to prevent the development of both a local and a general reaction Prophylactic immunization of this type seemed to diminish the incidence of scarlet fever among the inoculated As a result of these observations Gabritchewsky and his assistants were strongly of the opinion that streptococcus is the causative agent of scarlet fever

The therapeutic results obtained by the use of Moser's serum lent further support to this view Moser immunized horses to hemolytic streptococci obtained from the blood of patients suffering from scarlatina The serum thus prepared was used therapeutically and is said to have had marked beneficial effects causing a drop in the temperature and pulse, a diminution of the toxemia, early disappearance of the rash and a marked shortening of the duration of the disease Escherich who observed the work closely was much impressed by the therapeutic value of the serum and likened its action to that of diphtheria antitoxin Later antistreptococcic sera prepared by other investigators, however, failed to display the therapeutic efficiency of Moser's serum and created doubt in the minds of many concerning the usefulness of such sera

Much other evidence both for and against the etiological relationship of streptococcus to scarlet fever was presented at this time and as one weighs its importance in retrospect, the positive seems of more significance than the negative The outstanding objection, however, to the acceptance of streptococcus as the cause of scarlet fever remained the impossibility of differentiating satisfactorily this organism from hemolytic streptococci associated with the great variety of septic conditions As a result other etiologic agents were searched for Moser's serum dropped into disuse and streptococcus vaccine was no longer used in the prophylaxis of scarlet fever Scientific opinion gradually came to hold that streptococcus bore an important but secondary relationship to scarlet fever, and that the true cause must be sought among the unknown viruses

For many years confusion has existed and opinion has varied concerning the existence of biologically varying types of streptococcus Two diverging points of view developed, one maintaining the unity of the species as a type, and the other holding that it comprised a group of organisms different from one another in their biological characteristics Schottmuller (26) in 1903 made an important contribution to the discussion in demonstrating between certain streptococci, differences based on their action on blood agar plates, one group hemolyzing the red blood cells and the other either failing to hemolyze or forming methemoglobin This significant differentiation resulted in the establishment of the types now generally recognized as hemolytic and nonhemolytic or green pigment producing strains Further classification was attempted by numerous investigators who used as a basis of differentiation certain biochemical reactions Holman (27) in 1916 using carbohydrate fermentation as a test was able to demonstrate the existence of a number of separate fermentation types Numerous efforts were also made to establish biological differences, especially among the hemolytic streptococci, by means of serological reactions methods which had proven singularly successful when employed for studying the various types of pneumococcus and meningococcus As a result of these studies conflicting beliefs arose, and a definite opinion could not be given as to whether or not separate biological types of S hemolyticus exist As late as 1918 Swift and Kinsella (28) using the complement fixation reaction as a test made a series of observations of twenty-eight strains of hemolytic streptococcus from various sources They found that they were unable to determine significant serological differences between the strains studied and are of the opinion that a striking homogeneity exists Efforts to correlate such different types of hemolytic streptococcus as had been determined with specific pathological lesions were also of indeterminable significance, varying types being found in association with the same disease

In 1918 Dochez, Avery, and Lancefield (29) undertook a biological study of a great number of strains of S hemolyticus, obtained from a variety of pathological conditions among the changing population of a large military establishment The purpose of this investigation was to determine if there exist among the hemolytic streptococci diverse

biological types as is the case in the instances of pneumococcus and meningococcus The specific test reactions were those of agglutination and protection Spontaneous non-specific flocculation, the most confusing factor in previous studies of specific agglutination of streptococcus, was avoided by the employment of special methods The outcome of these studies was to prove that there are separate biological types among hemolytic streptococci, just as there are among other apparently closely related groups of microorganisms More than 68 per cent of the strains investigated comprised six easily distinguishable serological types

This study was part of a general investigation of the biology of streptococcus, and, as a result of the facts developed, Bliss and Dochez (30) undertook a reinvestigation of the much debated question of the unity of type of the S hemolyticus so constantly associated with scarlet fever An effort was made to answer Jochmann's main objection to the etiological relationship of S hemolyticus to scarlet fever, namely, that the organism is not present in every instance of the disease, and that it cannot be satisfactorily differentiated from hemolytic streptococci associated with the common septic conditions Bliss (31) found when cultures are made from the throat early in the course of scarlet fever that hemolytic streptococci are present in predominating numbers in 100 per cent of individuals examined, thus confirming the earlier work of Baginsky and Sommerfeld Immune sera were then prepared by the inoculation of rabbits with scarlet fever streptococci, and the capacity of these sera to agglutinate specifically a large number of freshly isolated scarlet fever strains was tested Ten such sera were prepared from different strains of scarlet fever streptococci and each serum was found to agglutinate more than 80 per cent of the strains isolated from scarlatinal throats Agglutinating sera prepared from strains of hemolytic streptococci derived from pathological sources other than scarlet fever in general, failed to agglutinate specifically the scarlatinal strains Furthermore, strains of hemolytic streptococci obtained from such conditions as tonsillitis, erysipelas, broncho pneumonia, and other septic diseases, as well as the various type streptococci, determined by Dochez, Avery and Lancefield were not agglutinated by the scarlatinal antistreptococcic sera The evidence in favor of the

specificity of the agglutination reaction of scarlatinal streptococci was reinforced by results obtained from agglutinin absorption experiments Scarlatinal streptococcic sera also afforded some protection of experimental animals against virulent scarlet fever streptococci, but had no protective power against hemolytic streptococci from other sources This work indicates that the majority of hemolytic streptococci found in association with scarlatina belong to a specific biological group and can by appropriate methods be distinguished from hemolytic streptococci derived from other pathological conditions These observations, I believe, confirm in a satisfactory manner the early studies of Moser and von Pirquet on the same subject Contemporaneously with Bliss, Tunnicliff (32) investigated, by means of the opsonic and agglutination reaction, a series of hemolytic streptococci isolated from patients during the early stages of scarlet fever She concludes that the serum of sheep immunized with hemolytic streptococci from the throat in the acute stage of scarlet fever contains opsonins and agglutinins for the hemolytic streptococci that prevail in the throat and complicating lesions early in this disease, but not for hemolytic streptococci from other sources, such as erysipelas, mastoiditis, measles, influenza, diphtheria and the normal throat The results of her absorption experiments also indicate that the hemolytic streptococcus from scarlet fever forms a distinct group, scarlatinal streptococci removing the opsonins and agglutinins for these cocci while absorption with a hemolytic streptococcus from erysipelas has no such effect These results also suggest that the hemolytic streptococci from scarlet fever form a distinct serologic group Somewhat later Gordon (33) found that eighteen strains of hemolytic streptococcus isolated from scarlatina were identical in their agglutinative reactions None of these strains absorbed the agglutinins from immune sera prepared from certain other types of hemolytic streptococcus, designated by him as Types I and II On the basis of this evidence, Gordon concludes that the streptococci from the throat secretions in scarlet fever constitute a group immunologically distinct from other varieties of streptococcus pyogenes Eagles (34) in a recent study compared the serological reactions of hemolytic streptococci from scarlet fever, puerperal sepsis, erysipelas and miscellaneous sources He confirms

the immunological specificity of the scarlatinal group and the clearness with which it can be separated from other types of streptococcus He furthermore compared in an interesting manner, a number of individual strains obtained at three to four day intervals from the same patient and demonstrated a gradual but progressive loss of specific agglutinability, a phenomenon which we have observed, and of which I shall say more later Williams (35) has also studied the serological reactions of the scarlatinal streptococci and finds only 35 per cent to belong to a single type, and is of the opinion that a greater variability exists than is suggested by the work of the previous observers Dick and Dick (36) have shown two strains of scarlet fever streptococci, one a mannite fermenter, and the other a non-mannite fermenter, to be serologically distinct, and believe that the agglutination reaction is of but little importance in determining the character of the scarlatinal streptococci

It would seem, therefore, that the old question stressed by Jochmann, concerning the specificity of the streptococcus of scarlet fever still remains in dispute The preponderance of evidence, however, strongly favors the belief that these cocci comprise a separate biological group and that the best method for determining this specificity of type is by agglutination In order that satisfactory results may be obtained from this reaction certain rigid conditions must be complied with, spontaneous autoagglutination must be prevented, and the streptococci in question must be studied fresh from their human environment This latter requirement is of great significance Recent studies by Avery and Heidelberger (37) have shown that the type specificity of pneumococcus is dependent upon the chemical constitution of the capsular substance The production of this substance is a variable function of the organism, it is greatest in its strictly parasitic phase and is reduced by all factors which reduce virulence and lessen pathogenicity That a similar loss of a specific function by scarlatinal streptococci takes place when they are removed from their parasitic environment is extremely likely Bliss and Stevens and Dochez (38) have emphasized the rapidity with which specific agglutinating qualities are lost upon continued growth of these streptococci in artificial medium and Eagles suggests that the same change may take place under the influence of the im

mune bodies formed by a scarlatinal subject during convalescen The suppression of specificity of serological reaction under the i fluence of immune bodies is of course a well recognized and esta lished phenomenon among the pneumococci The results obtain therefore indicate that if a large number of strains of scarlatinal stre tococci are studied under appropriate conditions and within a sho period of time from their isolation during the acute stage of scarl fever a high degree of serological specificity can be demonstrate

Streptococcus scarlatinae is found not only in the throats a organs of individuals suffering from anginal types of scarlet fever b has also been obtained from atypical forms of the disease, healtl carriers and contaminated food products Serologically speci streptococci have been isolated from the local lesions in scarlet fev arising from the infection of wounds and burns, from the throat scarlet fever without a rash, and from the lochial discharge in r stances of puerperal scarlet fever Bliss succeeded in tracing a sma outbreak of scarlatina in an isolated children's institution to a recent admitted healthy carrier of Streptococcus scarlatinae Stevens a myself identified by means of agglutination and absorption reactio as scarlatinal streptococci organisms isolated both from the co taminated milk which had given rise to a milkborne epidemic scarlet fever, and from the throats of patients who contracted th disease from this milk As a result of these studies the importan of Jochmann's objections to Streptococcus scarlatinae as the etiolo ical agent of scarlet fever was much lessened and students agai began to take an active interest in this organism as the probabl cause of the disease.

From the very beginning of the study of scarlet fever efforts hav been made to produce the disease experimentally in animals and i man by inoculation with scarlatinal material Most of these attempt have had in view the demonstration of an unknown virus of th filter passing type Streptococcus scarlatinae in spite of the pre sumptive evidence in its favor and of the fact that some of the mos typical examples of scarlet fever in animals have been associate with its presence has been but little tested for its capacity to produc the disease experimentally Class (39) in 1899 reported the experi mental production of this disease in swine by an organism designate

by him as Micrococcus scarlatinae This was a gram negative coccus isolated from three hundred cases of scarlet fever and was in all probability a streptococcus Krumwiede, Nicoll and Pratt (40) in 1914 observed an accidental infection of a laboratory worker, who sucked into her mouth a mixture of living streptococci containing Streptococcus scarlatinae Three days later this individual developed a sore throat and subsequently experienced a typical attack of scarlet fever with all the usual phenomena Because of the interest aroused by this observation, efforts were made to infect monkeys with the same streptococcus but no instance of the disease was successfully produced

In 1921 Dick and Dick (41) made a series of human inoculations with certain organisms obtained from the throats of individuals suffering from scarlet fever Among the organisms utilized for this purpose was Streptococcus scarlatinae Though some of the volunteers experienced sore throats as a result of the treatment no true instance of experimental scarlet fever resulted In 1923 the same workers (42) repeated their efforts to produce scarlet fever in human volunteers In the second series of observations a hemolytic streptococcus obtained from the infected finger of a nurse suffering from wound scarlet fever was used for purposes of inoculation Five volunteers were inoculated by swabbing the tonsils and pharynx with four-day old cultures of the streptococcus in question Three of these individuals remained without evidence of infection and one suffered from sore throat and fever without a rash The fifth volunteer, however, who had been inoculated with the streptococcus after three weeks growth in artificial medium experienced a typical but mild attack of scarlet fever, beginning forty-four hours after inoculation, and characterized by sore throat, general malaise, nausea, fever, leucocytosis a typical rash and albuminuria Desquamation began on the hands and feet on the tenth day and was complete by the end of the fourth week Five volunteers inoculated with filtrates of the above mentioned organism remained well and showed neither sore throat nor rash Subsequent inoculation of four of these volunteers with living unfiltered cultures of the original streptococcus resulted in the experimental production of another instance of scarlet fever These observations were confirmed later by the same inves-

mune bodies formed by a scarlatinal subject during convalescence. The suppression of specificity of serological reaction under the influence of immune bodies is of course a well recognized and established phenomenon among the pneumococci The results obtained therefore indicate that if a large number of strains of scarlatinal streptococci are studied under appropriate conditions and within a short period of time from their isolation during the acute stage of scarlet fever a high degree of serological specificity can be demonstrated

Streptococcus scarlatinae is found not only in the throats and organs of individuals suffering from anginal types of scarlet fever but has also been obtained from atypical forms of the disease, healthy carriers and contaminated food products Serologically specific streptococci have been isolated from the local lesions in scarlet fever arising from the infection of wounds and burns, from the throat in scarlet fever without a rash, and from the lochial discharge in instances of puerperal scarlet fever Bliss succeeded in tracing a small outbreak of scarlatina in an isolated children's institution to a recently admitted healthy carrier of Streptococcus scarlatinae Stevens and myself identified by means of agglutination and absorption reactions as scarlatinal streptococci organisms isolated both from the contaminated milk which had given rise to a milkborne epidemic of scarlet fever, and from the throats of patients who contracted the disease from this milk As a result of these studies the importance of Jochmann's objections to Streptococcus scarlatinae as the etiological agent of scarlet fever was much lessened and students again began to take an active interest in this organism as the probable cause of the disease

From the very beginning of the study of scarlet fever efforts have been made to produce the disease experimentally in animals and in man by inoculation with scarlatinal material Most of these attempts have had in view the demonstration of an unknown virus of the filter passing type Streptococcus scarlatinae in spite of the presumptive evidence in its favor and of the fact that some of the most typical examples of scarlet fever in animals have been associated with its presence has been but little tested for its capacity to produce the disease experimentally. Class (39) in 1899 reported the experimental production of this disease in swine by an organism designated

by him as Micrococcus scarlatinae This was a gram negative coccus isolated from three hundred cases of scarlet fever and was in all probability a streptococcus Krumwiede, Nicoll and Pratt (40) in 1914 observed an accidental infection of a laboratory worker, who sucked into her mouth a mixture of living streptococci containing Streptococcus scarlatinae Three days later this individual developed a sore throat and subsequently experienced a typical attack of scarlet fever with all the usual phenomena Because of the interest aroused by this observation, efforts were made to infect monkeys with the same streptococcus but no instance of the disease was successfully produced

In 1921 Dick and Dick (41) made a series of human inoculations with certain organisms obtained from the throats of individuals suffering from scarlet fever Among the organisms utilized for this purpose was Streptococcus scarlatinae Though some of the volunteers experienced sore throats as a result of the treatment no true instance of experimental scarlet fever resulted In 1923 the same workers (42) repeated their efforts to produce scarlet fever in human volunteers In the second series of observations a hemolytic streptococcus obtained from the infected finger of a nurse suffering from wound scarlet fever was used for purposes of inoculation Five volunteers were inoculated by swabbing the tonsils and pharynx with four-day old cultures of the streptococcus in question [illegible] of these individuals remained without evidence of infection [illegible] suffered from sore throat and fever without a rash [illegible] teer, however, who had been inoculated with the streptococcus [illegible] three weeks growth in artificial medium [illegible] mild attack of scarlet fever, beginning forty [illegible] tion, and characterized by sore throat [illegible] fever, leucocytosis, a typical rash and [illegible] began on the hands and feet on the [illegible] the end of the fourth week Five [illegible] of the above mentioned organism [illegible] sore throat nor rash Subsequent [illegible] teers with living unfiltered cultures [illegible] resulted in the experimental production [illegible] fever These observations were [illegible]

tigators (43) by the experimental production of another instance of scarlet fever in an individual proven susceptible by the use of a skin test devised by them

In 1920, Dochez and Bliss while studying the biological reactions of Streptococcus scarlatinae, observed in a dog infected subcutaneously with living organisms, the development of an intense general erythema followed later by desquamation Attempts to reproduce this phenomenon in dogs resulted in failure Stevens and Dochez later tried other animals, including monkeys without success Failure in these instances seemed to be due to our inability to induce a local infection because of the low virulence of the organism for the animals employed Finally Dochez and Sherman (44) were successful in producing in guinea pigs and young swine a series of manifestations comprising some of the principal phenomena of scarlet fever Successful local infection was achieved by injecting melted agar subcutaneously and infiltrating the mass with living cultures of Streptococcus scarlatinae Since it had become increasingly evident that scarlatina has a certain resemblance to diphtheria, in that there is a local infection in the throat from which the specific toxic substance is distributed, we hoped that a similar absorption of toxic material would take place from the local area of infected agar This proved to be the case and guinea pigs and swine treated in the manner described developed an erythematous rash, fever, leucocytosis and progressive loss of weight From eight to twelve days following infection the swine had general scaly desquamation and the guinea pigs slight general desquamation and complete separation of the skin over the pads of the feet This phenomenon could not be induced when hemolytic streptococci from sources other than scarlet fever were utilized Some of the guinea pigs died acutely from the toxic substances absorbed from the locally infected area, and after death streptococci could not be demonstrated by culture either in the blood or serous cavities

The production of experimental scarlet fever in human beings and in animals by inoculation with Streptococcus scarlatinae had by this time made it increasingly likely that this organism is the causative agent of the disease The evidence in favor of the absorption from the area of local infection of a toxic substance which might

be responsible for the clinical picture, had again brought into the foreground the analogy with diphtheria

Investigators of scarlet fever have for many years been impressed with the similarity of this disease to diphtheria Berge (45), as early as 1895, suggested that scarlatina is due to a local infection in the throat with streptococcus and that the general symptoms of the disease are due as in diphtheria, to the absorption into the general circulation of soluble toxins formed by the infecting microorganism at the site of the local disease Gabritchewsky and his co-workers, in their studies of scarlatiniform manifestations which followed immunization of human beings against scarlet fever by means of vaccines of killed cultures of streptococcus scarlatinae, attributed these reactions to the presence of a toxin in the vaccines They drew attention to the absence of a vaccine erythema in individuals who gave a history of having had scarlet fever, and its failure to develop in patients during the period of convalescence from this disease

Much evidence in favor of the existence of a soluble circulating poison in scarlet fever has also come from the study of the so called Schultz Charlton extinction phenomenon In 1918 Schultz and Charlton (46) discovered that if one injects into the skin of a scarlet fever patient with a bright red rash 1 cc of serum from a normal person, or from a patient convalescent from scarlet fever, there appears after a time at the site of the injection a characteristic change This change begins after about six hours and consists in a complete blanching of the rash over an area of from one-half inch to a few inches in diameter In the affected area the swollen follicles, which are a feature of many rashes, disappear Looked at from a distance the margin of the defect in the rash is generally sharply defined The colour of the blanched area is that of normal skin and the duration of the typical phenomenon coincides on the whole with that of the rash itself On the other hand, serum taken from scarlet fever patients during the acute stage of the illness invariably gave negative results Subsequent investigators abundantly corroborated the accuracy of the observation of Schultz and Charlton As a result of these later studies it was established that the serum of about sixty per cent of normal adults possesses the capacity to blanch the rash in an active case of scarlet fever, that convalescent scarlatinal serum

gives a positive rash extinction test in from 80 to 100 per cent of instances, and that the serum during the active stages of scarlet fever never manifests blanching power The Schultz-Charlton reaction was first used as a diagnostic test of scarlet fever, and the capacity to extinguish the rash in scarlet fever was believed to be due to a normal property of human serum, which is temporarily lost during the acute stage of scarlet fever and regained during convalescence

In 1923 Mair (47) published a study of the Schultz-Charlton reaction in which he confirmed in general the observations of previous workers but gave the phenomenon a much more satisfactory explanation He had an opportunity of studying the blanching power of the serum of a child both before and after an attack of scarlet fever and showed that the serum before the attack gave a negative Schultz-Charlton test but during convalescence acquired the capacity to extinguish an active rash This disproved the previous belief that a positive reaction was due to some property of normal human serum which is lost during the acute stages of scarlet fever He also showed that the sera of young children who had not had scarlet fever, give a negative reaction in a much greater proportion of instances than do adult sera and that the reactivity of the sera of newborn infants corresponds with that of the mothers

Mair had been interested for some years in the resemblance of scarlet fever to diphtheria As a result of his later work he came to believe that the rash and other changes in the skin in scarlet fever are due to a scarlatinal toxin which has entered into combination with the tissue cells Among the affected cells are those contractile elements which have been shown to exist even in capillary blood vessels, and to the function of which the normal tone of the capillaries is due The toxin interferes with the function of these cells and a loss of tone of the capillaries results in the erythema and exudative phenomena with which we are familiar in the scarlatinal rash He supposes that the serum giving a positive Schultz-Charlton reaction contains an antitoxin which is able to dislodge and neutralize the toxin fixed in the cells, and thus restores their normal function over the area injected He adds that the true causal organism when discovered should be capable of producing a toxin, and that the immunization of animals to this poison should give rise to an antitoxin capable of producing a positive Schultz-Charlton reaction in man

We also had been pondering over the analogy between scarlet fever and diphtheria, and, at the time of the publication of Mair's observations, had already produced in horses by immunization to Streptococcus scarlatinae an antitoxic serum of the type postulated by him Struck by the fact that occasionally in guinea pigs inoculated for the production of experimental scarlet fever sufficient poison was absorbed from the local lesion to kill the animals acutely, we determined to make use of the method for the production of an antitoxic serum in horses Masses of melted nutrient agar were injected beneath the skin and then infiltrated with increasing doses of Streptococcus scarlatinae The animals experienced a general reaction and some of them curiously enough showed loss of hair and extensive general desquamation After nine months immunization the first animal was bled and his serum tested by Blake and Trask and Lynch (48) for its correspondence with human convalescent scarlatinal serum When injected intracutaneously in a patient with a bright rash in the acute stage of scarlet fever this serum caused a complete extinction of the rash over an area five to ten centimeters in diameter The blanching appeared in from six to twelve hours following injection of the serum and persisted throughout the course of the disease As a rule the characteristic pigmentation and desquamation were absent during convalescence over the blanched area Antisera prepared from other hemolytic streptococci and from Streptococcus scarlatinae injected intravenously into animals, failed to induce blanching of the rash Furthermore, scarlatiniform rashes in such conditions as erysipelas measles, and other exanthematic diseases, were not influenced by the intracutaneous injection of the scarlatinal antitoxin Injection of a sufficient quantity of the serum intramuscularly in a patient in the exanthematous stage of scarlet fever causes a complete fading of the rash over the whole body in from twelve to twenty-four hours

Blake and Trask (49) have demonstrated that there is present in the circulating blood and in the urine during the acute stage of scarlet fever a toxic substance which causes an erythematous reaction when injected intracutaneously in individuals whose blood serum gives a negative rash extinction test This substance appears to be identical with the culture toxin of the Dicks and circulates in the

blood for several days When patients with scarlet fever having easily demonstrable amounts of this poison in the blood are injected with scarlatinal antitoxin, the circulating toxin is rapidly neutralized, a single dose of forty cubic centimeters causing its complete disappearance throughout the remaining course of the disease The antitoxin quickly predominates in the blood and the treated patients' serum acquires the capacity to induce a positive Schultz-Charlton extinction test, a property that does not develop in untreated patients until late convalescence The other toxic manifestations of the diseases are likewise favorably influenced An immune horse serum, therefore, prepared in the manner described, seems to contain a potent antitoxin and behaves in every way in a manner similar to human convalescent scarlet fever serum

The further studies of Dick and Dick (50) demonstrating the presence of a toxic substance in filtrates from blood broth cultures of Streptococcus scarlatinae have brought to light a number of new and important facts which develop still further the analogy between scarlet fever and diphtheria The toxic filtrate was obtained by these authors from a strain of streptococcus with which they had produced experimental scarlet fever in man When individuals who give a negative history for scarlet fever are injected intracutaneously with small amounts of this toxin, within about six hours there appears at the site of inoculation a small circular area of erythema, which increases in size and intensity of color for from eighteen to thirty-six hours Frequently the local reaction is accompanied by swelling of the skin When a series of normal persons who have not had scarlet fever are injected with this substance, 41 6 per cent of these show a positive erythema reaction in the skin, a manifestation resembling the Schick test for susceptibility to diphtheria The remainder who give a negative reaction are considered to be immune, because of the probable presence of circulating antitoxin in the blood, just as in the case of diphtheria In addition, patients who are recovering from scarlet fever when tested intracutaneously with this substance, give but a very faintly positive or uniformly negative skin reaction A similar condition of affairs is found to exist among those who have had scarlet fever at some earlier period of life If individuals who have been proven susceptible to scarlet fever by means

of the Dick test, are injected subcutaneously with larger amounts of the toxin, they exhibit certain of the toxic manifestations of the disease, such as nausea and vomiting, fever and an erythematous rash When toxic filtrate is mixed *in vitro* with a small amount of convalescent scarlet fever serum, its capacity to produce a positive skin reaction is completely neutralized Neutralization of the reaction was also obtained *in vivo* by the injection into susceptible human beings of larger quantities of convalescent serum More recent studies of the Dicks (51) have shown that individuals who react positively in the skin, can be immunized by repeated doses of the toxin, so that within a relatively short period of time the skin reaction becomes negative and there is some evidence to support the belief that such individuals may be immune to the disease scarlet fever

Zingher (52) in an extensive study has confirmed the observations of the Dicks and extended them somewhat He has shown that the Dick reaction is positive in the early stages of scarlet fever in most instances, and that it becomes increasingly negative as the disease progresses through convalescence He has, furthermore, drawn a very close analogy between the data obtained from the Schick test in diphtheria and those obtained from the Dick test in scarlet fever In general, susceptibility to the latter reaction is greater in childhood and diminishes in adult life There is also an inherited resistance to the toxin in infants whose mothers exhibit a negative reaction

These studies, therefore, indicate that there is present in sterile filtrates from cultures of Streptococcus scarlatinae a toxic substance which bears a specific relationship to scarlet fever By means of this substance it is possible to detect susceptibility in persons who have not suffered from scarlet fever, and furthermore to demonstrate the development of immunity in patients who are recovering from an attack of the disease This work brings further strong support to the belief that Streptococcus scarlatinae is the etiological agent of scarlet fever

In 1921, Di Cristina (53) in Italy, obtained from the blood of patients with scarlet fever an anaerobic Gram positive diplococcus Other Italian investigators subsequently isolated a similar organism

from the naso-pharynx, bone marrow, spleen and desquamating skin of children with scarlet fever This organism, on further study, was found to show specific serological reactions with the serum of recovered cases of scarlatina Inoculation of children with living cultures of the organism is said to have produced an attenuated form of scarlet fever Furthermore, prophylactic inoculation with killed cultures prevented the development of scarlet fever among a number of children exposed to the disease Unfortunately, we are not in a position as yet to determine with any assurance the significance of this organism in scarlet fever, since an opportunity to study it bacteriologically has not been afforded

Have we now reached the end of man's long struggle to find the cause of this interesting and at times formidable and dangerous disease? Personally, I think we have Belief that scarlet fever may be caused by a protozoan parasite, or by one of the mysterious ultramicroscopic viruses, must I think be discarded in view of the fact that the evidence brought forward in support of the causative relationship of such types of microorganisms to the disease is entirely unconvincing On the other hand, can we say with certainty that scarlet fever is caused by a type of Streptococcus hemolyticus? Certainly a chain of evidence in favor of this organism has been patiently and progressively forged which is as strong as that in many diseases whose etiology is now accepted without discussion The constant association of this organism with the primary and secondary manifestation of the disease, its specific character, its capacity to produce the experimental disease in man and in animals, the quality of human convalescent scarlet fever serum to neutralize the toxic effects of this streptococcus, the capacity of an antistreptococcus horse serum antitoxic in nature to counteract the specific toxic manifestations of the disease in man, and finally the isolation from Berkefeld filtrates of this streptococcus of a toxic substance which bears a specific relationship to immunity in scarlet fever, leaves little room to doubt that Streptococcus scarlatinae is the principal and probably only etiological agent of scarlet fever

Let us, therefore, be optimistic and assume that a just reward has come to those many soldiers in the army of science, too numerous to be mentioned in so short an exposition, and that another disease

has been added to those about which the essential specific facts are known Let us also hope that the methods of prevention and treatment based on these facts may prove as successful as the promising character of the preliminary work suggests

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HYDROGEN ION CONCENTRATION OF THE BLOOD IN HEALTH AND DISEASE

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INTRODUCTION

The following discussion of the theoretical and practical considerations involved in the measurement of the hydrogen ion concentration and its significance in body fluids is intended only as a convenient outline for those readers whose interests have been clinical rather than physiological The electrolytic theory is utilized in its simplest form, regardless of the present question of complete and partial dissociation and we use the familiar term hydrogen ion concentration rather than the less familiar though more accurate term, activity Only at one point in the paper have we used the term "activity", namely in referring to the Donnan equilibrium In this instance it seems to us of especial importance to keep in mind the distinction between thermo-

esses of digestion, respiration and excretion are all to a great extent concerned with substances which in solution dissociate into electrolytes Of immediate interest to this discussion are the acidic and basic substances which are present in the body fluids

Two points are of especial importance (1) that this reaction is a reversible one and that the undissociated molecules are in continual equilibrium with the ions, and (2) the ions as well as the undissociated molecules for the purposes of this discussion may be assumed, in the concentration met with in blood, to obey the law of mass action

Measure of acidity in solutions

In measuring the alkalinity or the acidity of any solution two factors must be considered, the *quantity* of the acidic or basic substance and the *intensity* of the degree of acidity or alkalinity The quantity factor is expressed in terms of the concentration of the substance in chemical terminology as the normality or the number of gram equivalents per liter

The *intensity* factor, the degree of acidity or alkalinity often referred to as the *reaction* of the solution, depends upon the H^+ and OH^- ion concentration and is expressed usually in terms of the normality of hydrogen *ions*

A most important property of aqueous solutions is that the concentrations of H^+ and OH^- ions are related to each other thus

$$[H^+] \times [OH^-] = K_w \qquad (2)$$

where for any given temperature K_w is a constant and when $[H^+]$ increases $[OH^-]$ decreases and vice versa, so that it is possible at any one temperature to express both acidity and alkalinity in terms of either ion It is usual to use the $[H^+]$ for this purpose and to speak of the hydrogen ion concentration of the solution Throughout this paper we will use a bracketed chemical symbol to indicate the concentration (or activity) of a substance in solution

The relation between the intensity and the quantity factor of acidity is analogous to the relation between the intensity and quantity factors in electrical energy Electromotive force is the intensity factor which determines whether or not current flows and the quantity factor, ampère, is the measure of the total amount of current This

relation may also be compared to differences between temperature and calories, or to the older analogy between the pressure of water, due to the height of the reservoir, and the content of the reservoir

Another interesting comparison between the intensity factors $[H^+]$ and t° is that the zone of $[H^+]$ compatible with life is as small compared to known $[H^+]$ concentrations as is the zone of temperature compatible with life to known temperatures

In biological solutions the normality of $[H^+]$ is so small that the fractional values are difficult to visualize Furthermore, in determining and plotting $[H^+]$ values it is often convenient to use logarithms Soerensen (1909) therefore introduced the term pH as a convenient symbol for a measure of $[H^+]$ concentration, defined as follows

$$pH = \log\left(\frac{1}{[H^+]}\right)$$

The relation of pH to $[H^+]$ is most clearly shown by examples An N/10 HCl solution, assuming it to be completely dissociated into H^+ and Cl^- ions, will also be tenth normal with respect to hydrogen ions, or

$$[H^+] = 1/10\,N = 0\ 1\,N = 1 \times 10^{-1}\,N$$

or

$$\text{its pH} = 1\ 0$$

In like manner the hydrogen ion concentration of a serum may be expressed thus

$$H^+ = 0\ 000{,}000{,}032\,N = \frac{0\ 32}{10^7}\,N = 0\ 32\,N \times 10^{-7}\,N = \frac{1}{10^{7\ 5}}\,N = 10^{-7\ 5\,N}$$

or

$$\text{its pH} = 7\ 5$$

In using pH it is necessary to remember that change of 1 pH unit indicates a 10-fold change in H^+ concentration, that increase in pH means decrease in $[H^+]$ and that pH 7 0 at 20° and 6 8 at 38° represent neutrality Thus a decrease of pH from 7 4 to 6 4 means that the H^+ concentration has become 10 times *greater*, and that at either 20° or 38° the solution instead of being slightly alkaline, has become slightly acid

Relation between [H+] and gram equivalent normality of acid concentration

Since [H+] is the measure of the *intensity* factor, and titration of the *quantity* factor, it follows that for any solution the difference between these two measurements is dependent upon the degree of dissociation With an almost completely dissociated acid, as tenth or hundredth normal HCl solution, the two measurements expressed in normalities agree but in the case of many acids as carbonic phosphoric, acetic, etc the dissociation is low and the [H+] normality is therefore only a small fraction of the gram equivalent normality of total acid concentration

In such solutions therefore the titration values give no indication of the actual [H+] concentration of the solution

Relation of [H+] to buffer effect

Let us now compare the effect of adding strong acid to solutions of salts of strongly and weakly dissociated acids If we add 1 cc of N/10 HCl to (1) a liter portion of 0 15 N sodium chloride (physiological saline) and (2) to a liter portion of 0 15 M sodium phosphate solutions both at neutrality (pH = 7), the resulting pH of the NaCl solution will become about 4, a 1000 fold increase in acidity, while the pH of the phosphate solution will be practically unchanged The importance in biology of substances, whose solutions show comparatively small changes in [H+] with large addition of acid or alkali was pointed out by L J Henderson (1908) and to them Soerensen (1909) gave the name "buffer" substances

The hydrogen ion concentration of body fluids in general and of the blood in particular is kept within rather narrow limits by means, in large measure, of such buffer systems In the blood the systems are in order of importance, hemoglobin acid and its salt, carbonic acid and alkali carbonate, serum proteins and their salts, and monobasic and dibasic phosphate In this discussion the proteins including hemoglobin will be considered to be dissociated as weak acids (see Loeb, 1922) The [H+] of a mixture of a weak acid with its salts may be calculated by the equation

$$[H^+] = K \frac{[\text{free acid}]}{[\text{salt}]} \qquad (3)$$

Thus for serum

$$[H^+] = K_1 \frac{[H_2CO_3]}{[BHCO_3]} = K_2 \frac{[BH_2PO_4]}{[B_2HPO_4]} = K_3 \frac{[\text{H protein}]}{[\text{B protein}]} \quad (4)$$

where K_1 K_2 K_3 represents the dissociation constants for the individual systems and B represents sodium or potassium

L J Henderson (1908) first showed that the carbonate and phosphate equilibria of this equation are applicable to the blood system In 1916, Hasselbalch used the equation in the logarithmic form and gave values for pK′ in the following equation

$$pH = \log \frac{1}{[H^+]} = \log \left(\frac{1}{K \frac{[H_2CO_3]}{[BHCO_3]}} \right) = \log \left(K' \frac{[BHCO_3]}{[H_2CO_3]} \right) = pK' + \log \frac{[BHCO_3]}{[H_2CO_3]} \quad (5)$$

Several relations, of especial importance in blood may be deduced from the above equations First, for a given $[H^+]$ the *ratios* of acid and salt components to each other are fixed for every system Secondly, if the ratio for any one system can be determined and if the K values are known the value of $[H^+]$ as well as of all the other ratios can be calculated This fact is the basis for the calculation of pH or of pCO_2 or $[H_2CO_3]$ when one of these and total $[CO_2]$ are known

A third fact is evident from (4) that $[H^+]$ is not dependent upon the concentration of the buffer substance but upon the ratio of concentration of its two components This is discussed more fully under "Alkali reserve"

The acid base balance of the blood

The reaction of the blood is maintained within narrow limits by a remarkable adjustment to varying conditions The daily metabolism involves the ingestion of varying quantities of acid and basic salts, the production in metabolism of tremendous quantities of acids and bases, espcially H_2CO_3 and NH_3 and the neutralization and excretion of these products The physiological and chemical mechanisms of these processes have been reviewed thoroughly recently by L J Henderson (1921), Van Slyke (1921a, 1921b), Barcroft *et al* (1922) and Wilson (1923) so that we need only refer briefly to factors which are of importance in abnormal conditions

Under normal conditions the reaction of the blood is stabilized

through three main processes in addition to the effects of its buffer excretion of the non volatile acids and bases through the kidney, change in the base binding properties of hemoglobin with oxygenation and reduction, and excretion of carbon dioxide through the lungs

The first process is slow compared with the almost instantaneous adjustments secured by the last two

The amounts of CO_2 formed in the tissue and transmitted to the circulating fluid is so great that if only the buffer action of the blood's buffer systems were available the resulting reaction would pass beyond the limits of acidity compatible with life However, oxyhemoglobin is a stronger acid than reduced Hb so that as Hb is reduced, which occurs coincidently with increase of CO_2, some of the base B bound with it as $BHbO_2$ may combine with H_2CO This process is reversed in the lungs, thus

$$\text{Tissue} \quad BHbO + H_2CO_3 \rightarrow BHCO_3 + HHb + O \qquad (6)$$

$$\text{Lung} \quad BHCO_3 + HHb + O \rightarrow BHbO + H_2CO_3 \qquad (7)$$

By such a mechanism the ratio of $\frac{H_2CO_3}{BHCO_3}$ is kept approximately constant (within 0 03 pH) and at the same time the transfer of oxygen is aided That each of these processes facilitates the other was pointed out by L J Henderson (1921)

From the viewpoint of acid base balance it is important that a certain amount of base is in a labile condition and can be exchanged between CO_2 and Hb in respiration

In the serum this interchange may take place directly, but since neither sodium nor potassium diffuses through the cell wall to any appreciable extent in this exchange, the exchange between cells and serum must be indirect It is accomplished by the transfer of Cl^- and H^+ ions between cell fluid and serum fluid Thus

$$\overbrace{KCl + HHb + O_2 \rightleftharpoons KHbO_2 + HCl}^{\text{cell}} \rightleftharpoons \overbrace{HCl + NaHCO_3 \rightleftharpoons NaCl + H_2CO_3}^{\text{serum}} \qquad (8)$$

Alkali reserve

The importance of the system $\frac{[H_2CO_3]}{[BHCO_3]}$ in serving as the first line of defence against abnormal acids lies in the ease with which excess

H_2CO_3 may be excreted through the lungs As has been pointed out in discussing equation (5) the concentration of the system $\frac{[H_2CO_3]}{[BHCO_3]}$ may be changed so long as the ratio is constant, without change in pH Thus whenever base is needed for neutralization of a stronger acid HX it can be furnished by the $BHCO_3$

$$BHCO_3 + HX = BX + H_2CO_3 \tag{9}$$

and the freed H_2CO_3 can be removed by increased respiration Excess of alkali may be met by the reverse process of decreased CO_2 elimination The $BHCO_3$ concentration in normal venous blood is maintained at between 45 and 60 vols per cent of CO_2 The base of this $BHCO_3$ constitutes the alkali reserve against abnormal acid The determination of the $[CO_2]$ of blood therefore serves as the most convenient means of measuring this alkali reserve Since the amount of total CO_2 thus bound is dependent upon the pCO_2, Van Slyke and Cullen (1917a) used the CO_2 capacity (the total CO_2 content after exposure to normal pCO_2) as the measure of the alkali reserve More recently Van Slyke (1921b) and the present authors have expressed the alkali reserve as the $BHCO_3$ content at constant pH

CO_2 absorption curves

The influence of the total buffer systems of blood is most clearly shown graphically by CO_2 absorption curves Two types of curves are generally used One is with $[BHCO_3]$ or total $[CO_2]$ as the ordinate and pCO_2 as abscissae Such a curve, first used by Bohr was employed in a very convenient graph by Haggard and Henderson (1919) giving both pH and $[H_2CO_3]$ values in addition

In such a graph the difference in $[BHCO_3]$ with change in CO_2 tension is the measure of the buffer exchange of base with change in pH

For many purposes a graph using $[BHCO_3]$ and pH as coordinates is preferable Such a diagram is that shown in figure 5 which is adopted from Van Slyke's (1921b) chart We have added to Van Slyke's diagram lines indicating CO_2 tension (Cullen and Jonas, 1923)

These curves indicate at once various conditions possible in the blood acid base system Henderson, Bock, Field and Stoddard (1924) have recently given examples of many possible combinations of curves useful in studying the blood electrolyte equilibrium

Influence of salts and proteins on the acid base equilibrium

The salt systems constituting the buffer systems in the blood comprise only one-fifth of the total salt concentration of the serum or blood The total salt concentration of normal serum is equivalent to a NaCl solution of from 0 79 to 0 81 per cent (0 13 to 0 14 N) The *variation* in neutral salt content in the serum and tissue fluids is probably not enough to materially affect the pH of the fluids through change in the salt effect upon dissociation Variation in the content of the proteins alters the buffer content of the blood but not significantly the various dissociation constants

However changes in total salt and protein concentration in serum and cell fluid cause shifts in the distribution of the ions, through the change in water content, in osmotic pressure and in the Donnan equilibrium

The Donnan theory of membrane equilibrium states that when two solutions a and b, separated by a semi-permeable membrane, contain both diffusible and non diffusible ions, the diffusible ions distribute themselves thus

$$\frac{[A]_a}{[A]_b} = \frac{[A']_a}{[A']_b} = \frac{[B]_b}{[B]_a} = \frac{[B']_b}{[B']_a} \tag{10}$$

Where A and A′ represent any two species of univalent anions and B and B′ any two species of univalent cations The brackets here indicate the thermodynamic activities of these ions, the ratios of which are related to but not identical with the ratios of their concentrations, a subject which will not be further discussed in this review Applied to the serum cell system we have

$$\frac{[Cl^-]_s}{[Cl^-]_c} = \frac{[HCO^-_3]_s}{[HCO^-_3]_c} = \frac{[H^+]_c}{[H^+]_s} \tag{10a}$$

The combined effect of water shift and Donnan equilibrium has been discussed by Warburg (1922) and by Barcroft *et al* (1922) More recently Van Slyke, Wu and McLean (1923) and Henderson, Bock, Field and Adair (1924) have added more data and discussed extensively the question of electrolyte equilibrium as applied to blood

Relation between pH of blood and serum

Blood is a heterogenous system of serum and cell phases Each of these phases, to be strictly accurate (Warburg, 1922, Van Slyke, Wu and McLean, 1923) may be subdivided into protein and water phases, but for this review we will consider blood only as divided into serum and cell phases.

Parsons (1917) pointed out that all pH measurements made on whole unhemolyzed blood were in reality measurements of pH of serum, and that all the values reported in the literature determined by electrometric methods on blood represented the pH of the serum of the reduced blood He further showed in association with Barcroft and others (1922) that the pH of the serum of completely reduced blood is about 0 05 pH less acid than the serum of the blood completely oxygenated at the same CO_2 tension This indicates a serum pH difference between ordinary venous and arterial blood of about 0 02 pH at the same pCO_2

Whenever the term "pH of blood" is used it must be understood to mean the pH of the blood serum

pH of blood cell

The blood cells are more acid than is the serum (Warburg, 1922, Van Slyke, Wu and McLean, 1923, Henderson *et al*, 1924) by from 0 08 to 0 14 pH at normal serum pH

Little is known of the actual change in reaction of the blood cells but it exerts an influence on electrolyte and water distribution between cells and serum With change in anion (Cl^- and HCO^-_3) and H^+ concentration, water is shifted between cells and serum thus changing the cell volume

Relation between [$BHCO_3$] of blood and serum

The [$BHCO_3$] of serum is higher than that of the cells and therefore higher than the [$BHCO_3$] of whole blood The determinations of total [CO_2] can be made on either whole blood or serum The relationship between the [CO_2] of serum and of whole blood is dependent upon the pH of the serum and upon the cell volume In considering the relation between [$BHCO_3$] of serum and cells it must be remembered

that Na and K ions under ordinary conditions do not diffuse between cells and serum The shift of base with change in oxygenation and reduction of hemoglobin in the cell results (see equation (8)) in a migration of Cl^-, HCO^-_3 and H^+ through the cell wall and in the serum an exchange of base between Cl^- and HCO^-_3 This changes the relative osmotic concentration in cells and serum and involves a water shift between serum and cells The relationship between serum and whole blood [$BHCO_3$] has been studied and reviewed recently by Warburg (1922), by Peters and his associates (1923, 1924) and by Van Slyke, McLean and Wu (1923)

Another relationship that is of interest is that between total [CO], the value actually determined by CO analysis, and [$BHCO_3$] The difference between these two quantities is commonly written as [$H CO_3$] in solution and can be calculated if either pCO_2 or pH is known This relation is discussed later

At normal pH of 7 3 to 7 4 the [$BHCO_3$] represents about 95 per cent of the total [CO_2]

In studying variation in the acid base equilibrium for clinical and physiological purposes, it is most accurate and convenient to use plasma or serum for both pH and [CO_2] since the influence of any changing cell volume is eliminated

pH of serum or plasma

For most studies of acid base balance, plasma from 0 3 per cent oxalated blood, and serum may be considered interchangeable, although, as pointed out by Warburg (1922) excessive amounts of oxalate shift the electrolyte between cell and serum and Hooper, Smith, Bell and Whipple (1920) demonstrated significant changes in cell volume arising from dry oxalate used as an anticoagulant However, plasma is perhaps preferable for colorimetric determinations (see Methods)

Regulation of pH of blood

The manner in which respiration, by increase or decrease of CO tension serves as a mechanism by which the reaction of the blood may be maintained within its normal range, is outlined above in the discussion of the buffer system The nervous control of this mechanism

is located in a respiratory center of the brain For many years it was thought that the CO_2 tension was the sole chemical factor that controlled respiration, then with knowledge of the constancy of the pH of the blood, and following the experiments of Hasselbalch and Lundsgaard (1912) it was generally accepted that it is the pH of the blood, which acting upon the respiratory center controls respiration More recently a different point of view has come to be held (Scott, 1918, Jacobs, 1920, Gesell, 1923, Cullen, Austin *et al*, 1923, Cullen and Jonas, 1923, Van Slyke, Hastings, Murray and Davies, 1924) The last mentioned workers conclude

"that when there spiratory mechanism is normal increase in alkaline reserve is only partially compensated by increase in CO_2 tension so that increase in pH also occurs In the same way decrease in alkaline reserve is accompanied by decrease in pH There is a decrease in CO_2 tension but not sufficient to prevent pH change The usual percentage change in hydrion concentration is about twice that in CO_2 tension The arterial CO_2 tension is kept normally between 35 to 45 mm, which is a much narrower range than would be necessitated for the maintenance of normal pH The conception of the CO_2 tension as a factor physiologically important only from its relationship to blood pH is not consistent with these facts When conditions force the organism to choose between change in CO_2 tension and change in pH it tends to compromise between the two, and acts in a manner to indicate that maintenance of normal CO_2 tension is in itself an important factor"

Possibly it is the reaction of the respiratory center as distinct from that of the blood, which is the important factor in control In addition disturbances in the nervous mechanism may change the response to chemical stimulation (J S Haldane, 1922) The normal physiological variation in serum between arterial and venous blood during respiration amounts to about 0 04 pH and about 7 mm of pCO_2 (see charts No 116 and No 119 of Henderson *et al* (1924))

DETERMINATION OF PH

The methods which have been used in studying pH of blood may be divided into three general groups The first group includes the electrometric methods which are based upon measurement of an electrometric

potential which is proportional to the H^+ concentration. The "gas chain" or "hydrogen electrode" and the "quinhydron" methods belong to this group

The second group includes the indirect method of calculating pH either from measurement of buffer ratio or after the method of Barcroft (1914) from change in the constant of Hill's equation for the equilibrium between oxygenated and reduced hemoglobin and the oxygen tension

The third group comprises the colorimetric or indicator methods which utilize indicators whose solutions give color effects dependent upon the pH

The principle of these methods are reviewed fully in Clark's (1920) book

The methods which have been of most value for investigation of blood pH are described briefly

Electrometric Hydrogen electrode

The method which has served as the reference method for all pH measurements is the hydrogen electrode or "gas chain" method which is based upon the fact that in a suitable cell the difference of potential between a metal electrode and a solution of its ions is proportional to the ion concentration (or in the newer terminology to the ion activity) Hydrogen gas, when absorbed by a platinum black electrode acts as a hydrogen electrode This method was used by Hasselbalch and Lundsgaard (1912) in establishing the first exact value of the pH of blood Parsons (1917) showed that the electrometric determination of pH of reduced whole blood really measures the pH of the serum of reduced blood Because of technical difficulties due to the presence of oxygen from the hemoglobin it is more accurate and convenient to use serum or plasma than blood With the technic now available an accuracy of 0 01 pH may be obtained (Donegan and Parsons (1919), Warburg (1922), Cullen (1922)) Although it is the method of ultimate reference, electrometric pH determination requires so much material, time, and experience that ordinarily it is to be used only in physiological studies It is of interest, however, that Cullen and Bulman find that with proper precautions, the quinhydrone electrode can be used with very small quantities of serum, although it can not be used with whole blood

Reference standard for pH determination

All hydrogen ion concentrations are referred to a hypothetical "normal hydrogen electrode" It is necessary to base the actual determination upon some reproducible standard of reference. The recent development in the knowledge of electrolytic dissociation and the use of the activity coefficient for hydrogen ion activity has led to some confusion The reference standard is relatively unimportant in any investigation where only changes of pH are being studied, but becomes of importance when comparing results from different laboratories on such questions as normal pH of blood

As pointed out later the relation between the pH at different temperatures is also confused in this question of reference standard

Clark (1920) and Soerensen and Linderstroem-Lang (1924) have agreed that all biological hydrogen ion concentrations shall be reported by them in terms of the older pH values based on conductivity instead of the newer pH activity values

They also agree that the standard of reference shall be the N/10 calomel cell to which they have assigned definite values at various temperatures The present authors prefer to standardize their pH determinations against a reproducible standard acid solution either N/10 HCl or N/100 HCl in N/10 KCl, and to assume no change in pH of this standard solution with change in temperature (see Cullen, Keeler and Robinson (1925))

With the latter standardization, Soerensen's phosphate standards have his values at 18° and are 0 03 pH less at 38°, i e , a phosphate solution which is 7 40 at 20° is 7 37 at 38°. The pK′ values for serum based on this system and using Bohr's α values are 6 18 at 20° and 6 10 at 38°, giving a temperature difference in agreement with other workers using the N/10 calomel cell standard

Calculation of pH from $BHCO_3$ content and CO_2 tension

This method using equation 5 requires determination of total CO_2 content, and of CO_2 tension The total $[CO_2]$ is determined most easily and accurately by Van Slyke's CO_2 apparatus (Van Slyke and Neill, 1924) and the pCO_2 may be determined either by alveolar CO_2 determination (when the subject can cooperate) or from the CO_2 absorption curve For the latter purpose the total CO_2 content is

determined on one portion of the blood or serum as drawn, and other portions are equilibrated with known CO_2 tensions at two or more points Analysis of total CO_2 of these equilibrated samples gives data for the CO_2 absorption curve Peters, Bulger and Eisenman (1924) have recently proposed the construction of the curve from one point (see our equations (16) and (17)) The intersection of this curve with the CO_2 content of the original blood or serum gives the CO_2 tension (pCO_2) as drawn If venous blood is used it is necessary to correct the total [CO] for the change in [$BHCO_3$] with change in oxygenation of hemoglobin (see equation (18))

This method, which has been extensively used requires appropriate pK′ values for blood and for plasma or serum The pK′ values for serum have been recently redetermined for serum, for a variety of clinical conditions (Cullen, Keeler and Robinson, 1925) The value of 6 10 at 38° and 6 18 at 20° are reliable for serum and plasma (see also Warburg (1922) for review of previous work)

The difference between pK'_{serum} and pK'_{blood} used to calculate the pH of serum since the pCO_2 is the same, must be dependent entirely upon the different solubility of CO_2 in whole blood and serum and upon the difference in [$BHCO_3$] This relation as pointed out above is influenced by cell volume (hemoglobin content) and pH of the serum This difference, pK'_B–pK'_s, has been recently studied by Warburg (1922), by Peters, Bulger and Eisenman (1924), and by Van Slyke, McLean and Wu (1923) The studies including that of Peters, Bulger and Eisenman on a large series of human bloods (see equation (17)) are in close agreement with each other Hastings (quoted by Van Slyke, Wu and McLean, 1923, p 800) has represented the relation on a D'Ocagne-Henderson line chart The values for pK′s we give in table 1, and for pK'_B–pK'_s in figure o

The technic which we employ for equilibration to known CO tension and for calculation of data is summarized in a previous paper (Austin *et al*, 1922)

Colorimetric determination of pH

Because of its simplicity and economy of material and time the direct determination of pH on blood plasma is the method of choice for clinical investigation The principle of the colorimetric method

involves (1) handling of blood and plasma without loss of CO_2, (2) either elimination of protein error by dialysis or correction for the protein error of the indicator The colorimetric and electrometric methods applied to protein free salt solutions are in perfect agreement when sufficient precautions are used to prevent loss of CO_2 (Cullen and Hastings, 1922)

The blood may be dialyzed against neutral saline solution and the pH of this dialysate determined by use of an indicator Levy, Rowntree and Marriott (1915) used this method and introduced the use of phenolsulphonephthalein (phenol red) ,as indicator These authors did not prevent loss of CO_2 so that their values did not represent actual pH Dale and Evans (1920) modified this method by preventing loss of CO_2 Dale and Evans used neutral red and titrated a phosphate control to the same color Lindhard introduced a micromodification of Dale's and Evan's method

Cullen (1922) determined directly the pH of the plasma diluted 1 20 with saline and determined by comparison with electrometric determination the total empirical correction for dilution, salt, protein and temperature This method has proved convenient for both clinical and physiological studies

Blood is drawn *without stasis or loss of* CO_2 into a tube under paraffin oil containing oxalate to make 0 3 per cent It is centrifuged in a stoppered tube completely filled with blood Especial care is taken that neither the blood or plasma is ever exposed to air One portion of plasma of from 0 2 to 1 cc is transferred to 20 volumes of 0 9 per cent NaCl solution containing phenol red, which is already covered with oil Another portion is added to 0 9 per cent NaCl solution without indicator The indicator NaCl solution is prepared by adding 1 05 cc of 0 04 per cent phenol red to 100 cc NaCl solution and adjusting with N/50 NaOH to pH about 7 5 Phosphate standards at 0 05 pH intervals are used containing 0 01 cc of 0 04 per cent phenol red per cubic centimeter The second sample of plasma in saline is used to superimpose the color of the serum upon that of the standard with indicator The color of the plasma + indicator tube is compared with the combined colors of phosphate + indicator and diluted plasma in a Walpole comparator For human plasma

$$pH_{38^\circ} = pH_{color} \text{ at } t^\circ + 0\ 01\ (t^\circ - 20^\circ) - 0\ 23 \qquad (11)$$

Where "pH_{38}" is the pH of the undiluted plasma at 38°, "t°" is the temperature (15° to 25°) of the phosphate standards and diluted serum when read, "pH_{color}" is the pH at 20° of the phosphate standard which matches the plasma + indicator tube

Hawkins (1923) has found that the whole blood can be added directly to the saline before centrifuging, thus 0 25 cc + 5 cc saline The empirical correction "C" given in equation 11 as −0 23 varies with different species and is apparently not identical in serum and in plasma and is perhaps more variable in the former The value for normal human plasma was found by Cullen (1922) to be −0 23 ± 0 04 The extent to which this correction varies under pathological conditions has not however been adequately determined Not only is the average value for other species different but the individual variation in some species, such as the dog, is apparently greater than in the human Hastings, Neill, Morgan and Binger (1924) found the average value for twelve pneumonia patients to be −0 27 ± 0 05 Recently Hastings and Sendroy (1924) report that reading both standard and diluted plasma at 38° eliminates this correction We have found this to be true in some sera but not in all For the present we must look upon the magnitude of this correction whether the reading be made at 20° or at 38° as open to further investigation *Calculation of CO_2 tension* may be made directly when total CO_2 and pH determinations have been made

Summary of equations and constants for expressing relations between certain factors in the acid base equilibrium

It seems desirable for convenience to gather together at this point certain relations between factors concerned in the acid base equilibrium in the blood and used in the calculation of one from another

The Henderson-Hasselbalch equation for the relation between pH of serum or plasma and CO_2 tension and CO_2 content of the serum or plasma is

$$pH_s = pK'_s + \log \frac{[BHCO_3]_s}{[H_2CO_3]_s} \tag{12}$$

This may also be written

$$pH_s = pK'_s + \log \frac{[CO_2]_s - \alpha_s pCO_2}{\alpha_s pCO_2} \tag{13}$$

involves (1) handling of blood and plasma without loss of CO_2, (2) either elimination of protein error by dialysis or correction for the protein error of the indicator The colorimetric and electrometric methods applied to protein free salt solutions are in perfect agreement when sufficient precautions are used to prevent loss of CO_2 (Cullen and Hastings, 1922)

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$$pH_{38°} = pH_{color} \text{ at } t° + 0\ 01\ (t° - 20°) - 0\ 23 \qquad (11)$$

approximation of the CO_2 absorption curve for human blood from the knowledge of the CO_2 content at one pCO_2

The level of true serum absorption curve above the blood curve at 38° has been found by them to be at $pCO_2 = 40$ mm

$$[CO_2]_s = [CO_2]_B + (0.0159\,[CO]_B - 0.281)\,h \tag{17}$$

Using equations (13), (14), (15) and figure o one can calculate $[CO]_s - [CO]_B$ for a given blood at any CO_2 tension and the result may be compared at $pCO_2 = 40$ with the value obtained by using equation (17) The calculated values for $[CO_2]_s - [CO]_B$ differ considerably, which is probably not surprising when the widely different kind of data on which the various constants are based is considered The discrepancy emphasizes the need for further experimental study of the relation between blood and serum already pointed out by the authors of these equations in order to define the applicability and limitations of the relations expressed in equations (14), (15), (17) and figure o The slope of the true serum curve may be approximated from that of the blood curve by assuming that $[CO_2]_s - [CO]_B$ is the same at 30 and 60 mm pCO_2 as at 40, or by assuming that $\log \frac{[BHCO_3]_s}{[H_2CO_3]_s} - \log \frac{[BHCO_3]_B}{[H_2CO_3]_B}$ is 0.01 less at $pCO_2 = 60$ and 0.01 more at $pCO_2 = 30$ than at $pCO_2 = 40$ The result obtained by either method is substantially the same and is approximately consistent with the observed relations The slope of the true serum curve can also be approximated by equation (16) for whole blood, given above

When the $[CO]_B$ and $[O]_B$ of blood as drawn is known and in addition the oxygen capacity and CO_2 absorption curve of the blood, fully oxygenated has been determined at body temperature in vitro and it is desired to determine the CO absorption curve of the blood at the state of oxygen saturation as drawn, we may use the formula of Doisy, Briggs, Eaton and Chambers at any pH (pH = X)

$$\frac{[CO_2]_B - [CO_2]_{sat}}{[O_2]_{sat} - [O_2]_B} = 0.41 \tag{18}$$

where $[O_2]_{sat}$ = oxygen capacity

$[O]_B$ = oxygen content in the state of partial unsaturation

For this purpose we follow Van Slyke, Wu and McLean (1923) using their equation (30) This gives

$$\alpha_B = (1 - 0\ 0067\ h)\ \alpha_s \tag{15}$$

Where h = oxygen capacity in volumes per cent Using equation (15) to express the relation between α_s and α_B we take as values for $pK'_B - pK'_s$ the graph, figure 6b, from Van Slyke, Wu and McLean (1923) These values they point out agree with the data of Peters, Bulger and Eisenman (1923)

By the courtesy of Drs D. D Van Slyke and A B Hastings we include a revised form of this graph as figure o

The use of CO_2 content and CO_2 tension of whole blood and equation (14) to calculate pH_s is open to much more uncertainty in the values of the constants of the equation than is the use of CO_2 content of serum and equation (13) The latter course is to be preferred wherever possible, this is especially true when blood from different species is under study

In the study of the carbon dioxide absorption curve of blood we formerly required a knowledge of the CO_2 content of a given sample of blood at three known CO_2 tensions or at three pH values in order to plot the CO_2 absorption curve Subsequently, however, it was shown by L J Henderson (1921) and Warburg (1922) that with changing pCO_2, $[BHCO_3]$ of blood plasma plotted against its pH is approximately linear Barcroft, Bock, Hill, Parsons, Parsons and Shoji (1922) showed that under the same conditions $[CO_2]$ plotted against $[H^+]$ is approximately linear and Peters, Eisenman and Bulger (1923) showed that under the same conditions log $[CO_2]$ plotted against log pCO_2 is linear Each of these methods make possible the determination of the CO_2 absorption curve from a knowledge of CO_2 content and either pCO_2 or pH at two CO_2 tensions Finally for human blood Peters, Bulger and Eisenman (1924) have shown that the slope of log $[CO_2]$ against log pCO_2 can be approximated if the oxygen capacity be known, as follows

$$\Delta\ [CO_2]_{60-30} = 0\ 334\ h + 6\ 3 \tag{16}$$

where "$\Delta[CO_2]_{60-30}$" is the increase in CO_2 content in volumes per cent between $pCO_2 = 30$ mm and $pCO_2 = 60$ mm , and "h" is the oxygen capacity expressed in volumes per cent This permits the

approximation of the CO_2 absorption curve for human blood from the knowledge of the CO_2 content at one pCO

The level of true serum absorption curve above the blood curve at 38° has been found by them to be at $pCO_2 = 40$ mm

$$[CO_2]_s = [CO_2]_B + (0.0159\,[CO_2]_B - 0.281)\,h \qquad (17)$$

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When the $[CO_2]_B$ and $[O_2]_B$ of blood as drawn is known and in addition the oxygen capacity and CO_2 absorption curve of the blood, fully oxygenated has been determined at body temperature in vitro and it is desired to determine the CO_2 absorption curve of the blood at the state of oxygen saturation as drawn, we may use the formula of Doisy, Briggs, Eaton and Chambers at any pH (pH = X)

$$\frac{[CO_2]_B - [CO_2]_{sat}}{[O_2]_{sat} - [O_2]_B} = 0.44 \qquad (18)$$

where $[O_2]_{sat}$ = oxygen capacity
$[O_2]_B$ = oxygen content in the state of partial unsaturation

$[CO_2]_{sat}$ = CO_2 content of blood, at pH = X, when saturated with oxygen

$[CO_2]_B$ = CO_2 content of blood, at pH = X, when at the oxygen saturation indicated by $[O_2]_B$

$[CO_2]$ and $[O_2]$ must be expressed in the same units (volumes per cent, mM , etc) The CO_2 absorption curve of the oxygenated blood can then be raised at each pH to a level corresponding to that for the state of unsaturation as drawn and the $[CO_2]_B$ as drawn interpolated on this curve to obtain the pH as drawn, whence the pCO_2 can be calculated

Logarithmic paper ruled as suggested by Peters (1923) with pH lines is very useful in plotting CO_2 absorption curves Such a chart can be prepared for serum but not to advantage for whole blood since for its preparation a constant value for α is necessary when pCO_2 is used as abscissae and also a constant value for pK′, but both α_B and pK'_B vary in different bloods according to the oxygen capacity

Temperature effect

In the biological studies thus far made, interest has centered almost exclusively upon the cause and effect of changes in $[H^+]$ under conditions of constant temperature or where at least change in temperature was not an essential feature of the conditions being investigated This has been of importance, sometimes unrecognized, in view of certain limitations in the measurements of $[H^+]$ and in view of the relation between $[H^+]$ and $[OH^-]$

For electrometric measurement of $[H^+]$ at any given temperature a value for $[H^+]$ of some standard solution must as we have already pointed out be more or less arbitrarily assigned for the temperature in question; then at the same temperature the ratio to this of $[H^+]$ of any other solution having been measured, its $[H^+]$ can be calculated with reference to the standard chosen The various standards in use in this connection we have already discussed

The direct measurement with the gas chain of an unknown $[H^+]$ at one temperature against a standard $[H^+]$ at another temperature is without any physical significance and cannot be interpreted For each temperature employed with the gas chain a new value for the $[H^+]$ of the standard solution or cell must therefore be assumed, or calcu-

lated in accordance with some assumption, and no one basis for this assumption has been generally accepted in biological work. The relation of pH = 7.40 at 20° to pH = 7.40 at 38° is one, therefore, that can be stated only when one has defined the standard values employed at each temperature.

The difficulty just stated is one of standardization of measurement. There is, however, a second factor to be considered in dealing with varying temperatures. In aqueous solution $[H^+] \times [OH^-] = K_w$ and at any given temperature, K_w is constant. At constant temperature any increase in acidity always signifies a proportionate decrease in alkalinity. In considering the biological significance of a change of reaction we as a rule make no attempt to distinguish the importance of change in $[H^+]$ and $[OH^-]$ respectively, and at constant temperature such a distinction is of no great significance since they are inversely proportionate.

When we deal with changing temperature the situation alters, however. With change in temperature K_w changes. With change in temperature it would be possible to have simultaneous increase in both $[H^+]$ and $[OH^-]$, change in one no longer implies a reciprocal change in the other. Under these conditions in considering reaction we have three distinct factors we may consider:

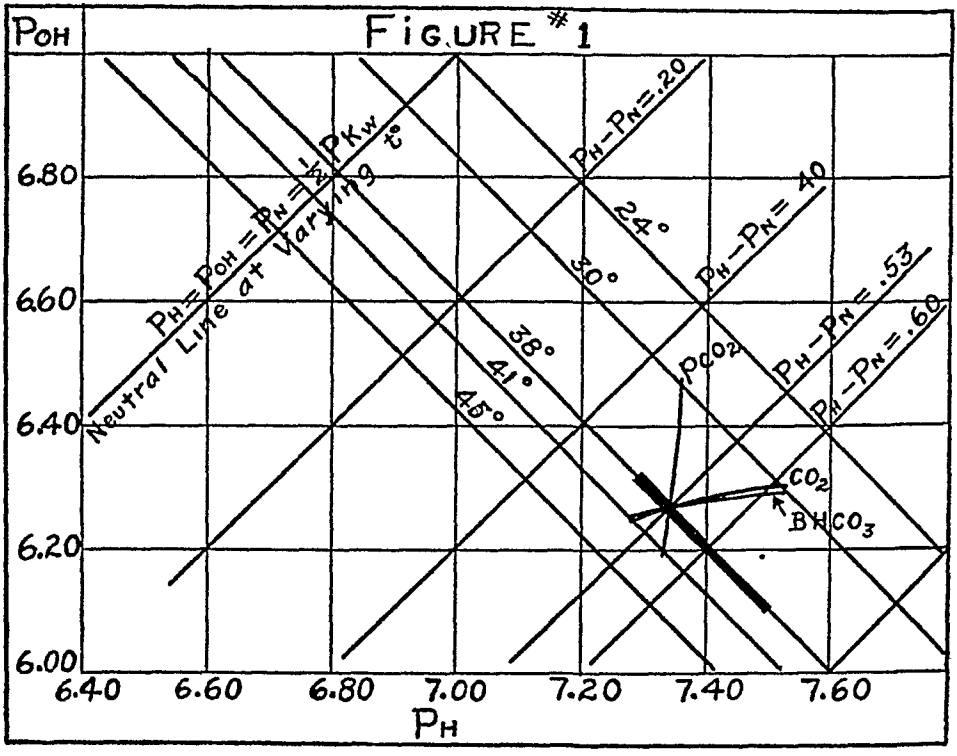
$$(1)\ [H^+],\ (2)\ [OH^-],\ (3)\ \frac{[H^+]}{[OH^-]}$$

When the ratio of $\frac{[H^+]}{[OH^-]} = 1$, the condition is by definition one of neutrality.

The values for K_w, that is for the product $[H^+] \times [OH^-]$, at different temperatures have been calculated by Lewis and Randall (1923, p. 487) on the basis of Wormann's determinations of heats of neutralization of strong acids and alkalies at various temperatures. Their values have been used in construction of figure 1, which illustrates the relationships of $[H^+]$ and $[OH^-]$ at varying temperatures. It will be seen from figure 1 that a serum which at 38° has a pH of 7.40 has the same ratio of $\frac{[H^+]}{[OH^-]}$ and a pH equally removed from the neutral point as a serum at 41° with a pH of 7.36.

In biological studies under conditions of varying temperature we

must determine, therefore, whether the significant factor is $[H^+]$ or $[OH^-]$ or the ratio of $\frac{[H^+]}{[OH^-]}$ and our logarithmic notation will be accordingly pH or pOH or 1/2 [pH−pOH], the latter expression measuring the increment of pH to the acid or alkaline side of the neutral point at the temperature in question Clark (1920) has pointed out that the neutral point although a convenient point of reference is



pH AGAINST pOH AT VARYING TEMPERATURES

The line of neutrality where pH = pOH at varying temperature

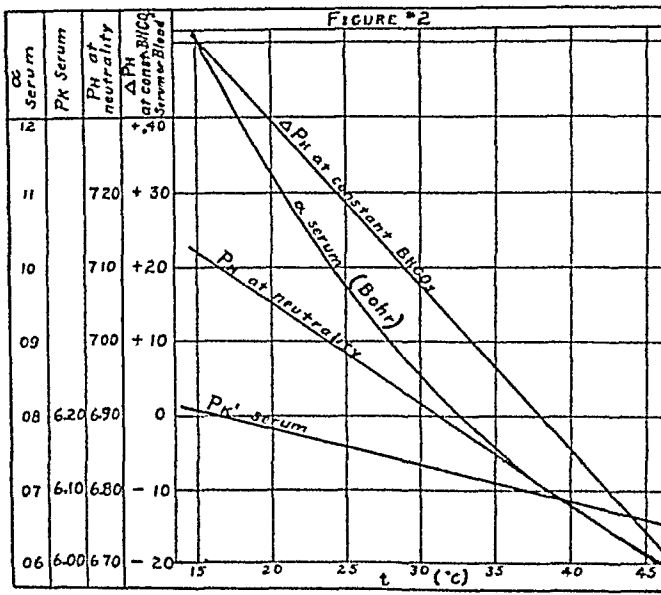
The shaded area is the normal pH, pOH and temperature range of serum

The curves show the effect of changing the temperature of a particular blood and its true serum at constant pCO_2, at constant $BHCO_3$ and at constant total CO_2

not as a rule characterized by any striking change in chemical behavior We may, however, expect to find in some reactions the major importance attaching to pH and in others to pOH

In the study of blood acid base equilibrium under conditions of varying temperature there are four variables of which the temperature coefficient must be taken into account.

1 The solubility of CO_2 The solubility of CO_2 in serum is given in table 1 and figure 2 So far as we know equation (15) is applicable at any temperature under consideration



(1) α-serum in equation

$$[H_2CO_3]_s \text{ (vols per cent)} = \alpha_s pCO \text{ (in millimeters)}$$

using Bohr's data

(2) PK′ serum in equation

$$pH = pK'_s + \log \frac{[BHCO_3]_s}{[H_2CO_3]_s} = pK'_s + \log \frac{[CO_2] - \alpha pCO_2}{\alpha pCO_2}$$

using Bohr's α,

(3) $pN = pH$ at neutrality $= pOH$ at neutrality $pOH = 2pN - pH$

(4) Δ pH at constant $[BHCO_3]$, permits construction of CO_2 absorption curve at changed temperature

2 The value of pK'_s at varying temperatures has been recently determined by Cullen, Keeler and Robinson (1925) and the result of their observations is given in figure 2 The observed change with

absorption curves in figure 3 represent the approximate calculated change in the true serum from the blood of W C S from the data of Stadie and Martin (1924). It will be seen that when the blood temperature is increased from 38° to 41° and the CO_2 tension is kept constant the following ensue

Slight reduction of pH (slight increase of $[H^+]$)

Marked reduction of pOH (marked increase of $[OH^-]$)

Marked decrease in the ratio $\frac{[H^+]}{[OH^-]}$

Decreased CO_2 capacity

TABLE 2

Effect of change in temperature on pH of true serum and separated serum various factors being kept constant

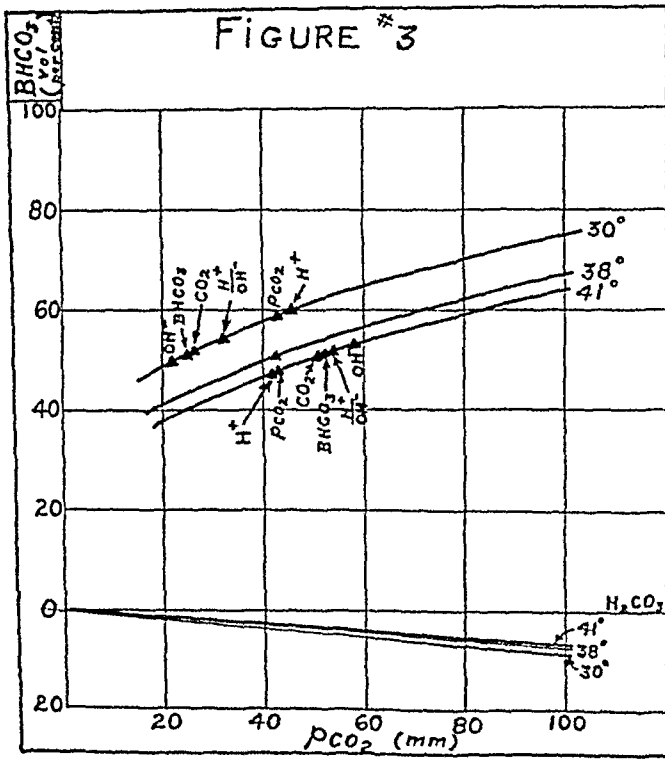
(Calculated from equations (19) and (5))

TEMPERATURE	ASSUMED CONDITION	$[CO_2]$	pCO_2	$[H_2CO_3]$	$[BHCO_3]$	pK'	pH	ΔpH	log R
	True serum $\frac{d[BHCO_3]}{dpH} = -62$ (t° constant)								
		vols per cent	*mm*	*vols per cent*	*vols per cent*				
38°	Initial	54 1	43 3	3 07	51 0	6 100	7 320		1 220
41°	Constant $[BHCO_3]$	54 4	51 2	3 41	51 0	6 085	7 260	−0 060	1 175
41°	Constant pCO_2	51 0	43 3	2 88	48 1	6 085	7 308	−0 012	1 223
41°	Constant $[H_2CO_3]$	52 3	46 0	3 07	49 2	6 085	7 290	−0 030	1 205
	Separated serum $\frac{d[BHCO_3]}{dpH} = -14$ (t° constant)								
38°	Initial	54 1	43 3	3 07	51 0	6 100	7 320		1 220
41°	Constant $[BHCO_3]$	54 4	51 2	3 41	51 0	6 085	7 260	−0 060	1 175
41°	Constant pCO_2	53 0	43 3	2 88	50 1	6 085	7 325	+0 005	1 240
41°	Constant $[H_2CO_3]$	53 5	46 0	3 07	50 4	6 085	7 300	−0 020	1 215

Under these conditions fall in pH (increase in $[H^+]$) does not mean diminished alkalinity.

These relations are also shown in figure 1 It will be seen that change in temperature at constant pCO_2 causes little change in pH but marked change in pOH On the other hand change in tem-

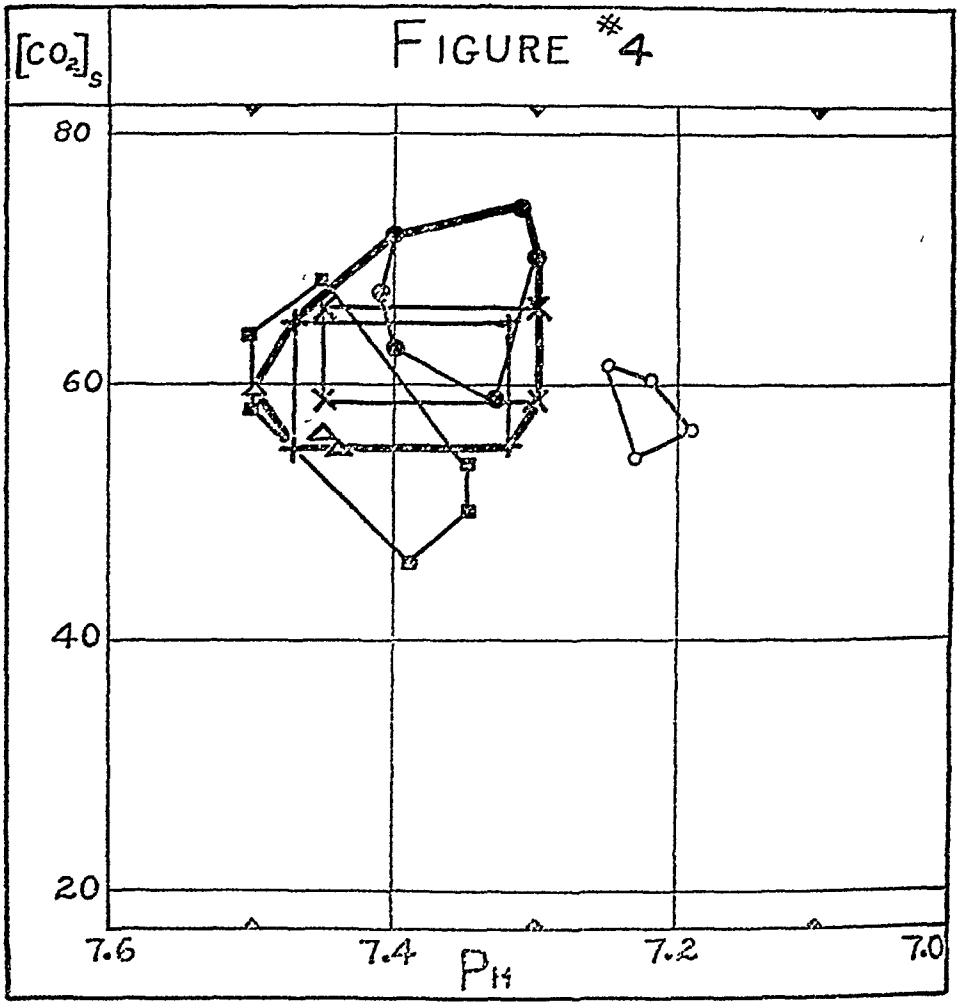
perature at constant $[CO_2]$ or $[BHCO_3]$ causes marked change in pH and little change in pOH These data permit us to apply our methods of calculating to the case of febrile blood or to blood from chilled or heated extremities



THE CO_2 ABSORPTION CURVES FOR TRUE SERUM OF THE SAME BLOOD AT THREE TEMPERATURES ARE SHOWN

An initial point is indicated on the 38° curve and on the other curves are indicated the points which have the same pH, pOH, $p\frac{(H^+)}{(OH^-)}$, $[BHCO_3]$, $[CO_2]$ and pCO_2 as the initial point

When blood is obtained at a temperature other than 38° and its $[CO_2]$ determined and in addition its CO_2 absorption curve at 38° is determined the following corrections must be introduced to determine pCO_2 and pH as drawn



NORMAL PLASMA $[CO_2]$ AND pH VALUES

•——• Cullen and Robinson (1923)

x——x Marrack and Boone (1923)

+——+ Myers and Booher (1924)

Δ Koehler (1923)

⊠——⊠ Hastings, Neill, Morgan and Binger (1924), $[CO_2]s$ values raised from blood to serum values but not corrected from arterial to venous values

○——○ Chambers and Kleinschmidt (1923)

▬▬ Values taken as normal for purposes of this review

1 Relocation of curve for unsaturation (equation (18))
2 Relocation of curve for temperature (equation 19 or Δ pH at constant $BHCO_3$ from figure 2)
3 Use of α_s pK′, and pN for appropriate temperature (see figure 2)

When blood is obtained at a temperature other than 38° and its [CO] determined and its pH at 38°, the correction to original pH is given by equation 19 This equation is at present an approximation only and can therefore be used for constant $[CO_2]$ as well as for constant $[BHCO_3]$

NORMAL pH VALUES

pH of normal serum and plasma

The range of normal variation in serum pH was given by Van Slyke (1921b) as pH 7 3 to 7 5 Cullen and Robinson (1923) studying normal students with the colorimetric method found a variation from 7 28 to 7 41 All but two, 7 28 and 7 41 fell within the limits 7 40 to 7 30

Bigwood (1923) with another series with the same method confirms this variation of 7 30 to 7 10 Myers and Booher (1921), using the same dilution but reading the determination in Myers colorimeter found the limits of normal pH to be between 7 35 and 7 43 However, Kochler (1923) and Marrack and Boone (1923) found somewhat higher normal pH values as do Hastings, Neill, Morgan and Binger (1924) in arterial blood The values of Chambers and Kleinschmidt (1923) are much lower and must represent a different pH standardization We have taken for this review the normal outline indicated in figure 4 with pH range from 7 30 to 7 50

In the observations of Cullen and Robinson the zone 7 30 to 7 40 was found normal not only for various individuals but for a single individual That is, the pH of any individual might on successive days vary between 7 30 and 7 10

Drucker and Cullen have developed a technique for taking capillary blood from the heel of infants or the ear of adults without loss of CO using Hawkin's modification of Cullen's method for pH and employing 0 25 cc of blood They find the pH of normal infants' capillary blood to be between 7.34 and 7 41 Since the capillary blood has been shown by Lundsgaard to approximate arterial blood in

composition its pH will be about 0 03 more alkaline than venous blood The range observed, therefore, is similar to that found by Cullen and Robinson for adults

pH of body fluids other than serum

As stated by Van Slyke (1921b) our limited knowledge of body fluids indicates that extracellular fluids have approximately the same pH as serum Parsons and Shearer (1920) report that the pH of cerebrospinal fluid is that of normal plasma pH. Boots and Cullen (1922) found in nonpurulent joint exudate in rheumatic fever pH values of 7 3 to 7 4 In this laboratory we observed the same pH for edema fluid and blood serum

pH of purulent fluids. Whenever the pH of purulent fluids has been measured it has been found more acid than the serum In purulent joint exudates Boots and Cullen (1922) found pH of 6 40 Lord has reported pH of 6 8 to 6 2 in consolidated portions of lungs in lobar pneumonia This range has also been observed by Avery and Cullen (unpublished)

Intracellular pH. Little is known of intracellular reaction other than that of the red cells which is estimated by Warburg (1922) and by Van Slyke, Wu and McLean (1923) for normal serum pH of 7 4 to be 0 08 to 0 14 pH more acid than serum Probably other intracellular fluids vary, at least as much from that of serum

pH of secretions It is interesting that the cells of the body although bathed with fluid of such constancy of pH as serum, can function in contact with a very high degree of acidity The pH of gastric juice may be less than 2 The pH of urine ranges from 5 0 to 8.5, duodenal fluid may have a pH over 8 0 Saliva has a normal pH of 6.7 (Starr, 1922)

pH OF PLASMA IN DISEASE

Abnormal acid base balance

The terms acidosis and alkalosis, common in clinical usage, are often used by different individuals to describe entirely different conditions Because the first recognition of abnormal acid base balance came through the knowledge that in diabetes abnormal metabolism resulted in the excretion of aceto-acetic acid, β-oxybutyric acid and acetone,

the term acidosis is often used to describe either a ketonuria or a ketonemia The term "acidosis" has been applied to the following conditions, (1) acetone bodies in the urine (2) acetone bodies in the blood, (3) decreased alkali reserve, (4) decreased CO_2 tension of alveolar air, (5) and decreased pH of plasma

This confusion is well recognized in physiological work and various attempts have been made to establish a more precise terminology Van Slyke (1921b) in his review of abnormal and normal acid base balance divided the possible conditions of abnormality into six groups which are best visualized from his diagram which is used with slight relocation of the normal area as the basis of figure 5

The Report on Acidosis of the British National Research Council, proposes the terms alkalosis and acidosis to indicate high and low levels of $[BHCO_3]$ and alkalemia and acidemia to indicate high and low pH Hasselbalch, Haldane, Van Slyke and the present authors (in studying the acidosis of anesthesia) have used "true acidosis" to mean coincident abnormally low pH and $[BHCO_3]$

It is probable, however, that no general usage for the term acidosis can be agreed upon and it is therefore better to avoid the term wherever possible or else to state the exact sense in which it is used

For the present it is best to state the conditions of the acid base balance in terms of two of its factors, either in terms of Van Slyke's nine areas, or perhaps simply as an acid base condition of 7 4 and 50, indicating $pH_a = 7.4$ and $[CO_2]_a = 50$ volumes per cent The term "A B C (acid base condition of 7 4 and 50)" might supply the need for an easily handled term The nature of the coordinates 7 4 and 50 make confusion between them impossible Whatever the terminology, it is evident that determination of only one of the three variables pCO_2, pH or $[CO_2]$ is not sufficient to determine the acid base condition

The studies of the alkali reserve of the blood in disease are much more numerous than those dealing with direct measurement of the pH of the plasma Simultaneous studies of CO_2 tension and CO_2 content of the plasma in disease from which the pH can be calculated are also rare

Accumulation of acids other than HCO_3^- in the blood with depletion of the blood bicarbonate has been described as a rule as occurring at

first without change in pH and only after considerable reduction in the bicarbonate to be associated with a more acid serum pH Such a course of events is approached in the acidosis of fasting, diabetes and nephritis in figure 5 The compensated stage of alkali deficit was believed to be brought about through a response of the respiratory center to an unmeasurably small diminution in pH with consequent stimulation of pulmonary ventilation, and consequent diminution in pCO_2 in alveolar air and arterial blood just sufficient to secure an almost proportionate reduction of bicarbonate and of CO_2 tension with almost constant pH

Only when the respiratory mechanism fails to function adequately and to compensate was change in pH believed to occur With the introduction of Cullen's (1922) method for direct measurement of the pH of serum or plasma as drawn and the application of this method to disease we are recognizing, however, that fall in plasma bicarbonate due to introduction of other acids, is often associated throughout its course with rise and fall of pH as indicated by the numbered arrows in figure 5 Evidence for this is to be found in the studies we describe of acid and alkali administration, of anesthesia, of exercise and radiation This behavior of blood reaction has a bearing upon the studies of the regulation of the respiratory center and the relative importance of pH and of pCO_2 in this regulation This has been previously discussed

Excessive introduction of acids

Many experimental studies have been made upon the effects of acid fed by mouth or administered intravenously The effect, we have observed in the dog, from injecting HCl, H_3PO_4, NaH_2PO_4 and lactic acid is shown in table 3. These injections were made under local anesthesia into the vein (in one case into the heart) using M HCl, M/10 H_3PO_4, M/10 NaH_2PO_4 and M/1 lactic acid

The change in pH and [$BHCO_3$] is shown in table 3 The direction and magnitude of the change in the acid base equilibrium is shown by the numbered arrows in figure 5

The apparently less marked effect of H_3PO_4 on the [$BHCO_3$] and its greater effect on pH is due to the large amount of acid injected leading to almost complete disappearance of $BHCO_3$ from the blood The

less pronounced effect of NaH_2PO_4 both on bicarbonate and pH is to be expected The absence of effect of lactic acid on pH is, however interesting At the pH of blood the ratio of $\frac{\text{lactic acid}}{\text{sodium lactate}}$ is about $\frac{1}{3200}$, taking the dissociation constant of lactic acid as 1.38×10^{-4} (Landolt-Bornstein 4th edition p 1147) The markedly diminished effect of lactic acid per millimole on the bicarbonate is compared with HCl is therefore not to be expected except as a result of disappearance, of the lactic acid as for instance, by synthesis to glucose or as a result of its wider distribution in the body fluids as in the case of bicarbonate, later described

TABLE 3

ACID	CONCENTRATION	AMOUNT INJECTED PER KILO	TIME OF INJECTION	Δ[HCO₃] PER MILLIMOLE OF ACID PER KILO	Δ[H] PER MILLIMOLE OF ACID PER KILO
	M	mM	minutes	mM	
HCl	1.0	2.2	10	−6.0	−0.11
HCl	1.0	2.2	10	−7.0	−0.06
HCl	1.0	1.1	6	−6.0	−0.06
H_3PO_4	0.1	4.9	49	−4.0	−0.16
NaH_2PO_4	0.1	4.9	52	−2.0	−0.03
Lactic	1.0	2.2	6	−2.0	+0.01

When HCl is given by mouth the change in plasma bicarbonate and pH depends on the relative rate of absorption and of compensating excretion In the observation of Gamble and Ross (1923) the administration to a child of 17 mM per kilo of HCl in 2 days lowered the plasma bicarbonate 9 mM and the pH 0.17 Haldane (1923) and Gamble and Ross (1923) pointed out that the ingestion of ammonium chloride in man causes marked and prolonged acidosis, due presumably to conversion of part of the ammonia into urea, thus freeing the acid which had been combined with it The acidosis is shown by fall in the plasma CO_2 capacity and fall in the alveolar pCO_2 There is a roughly commensurate increase in the rate of acid plus ammonia excretion in the urine, the ratio between the increase in acid and increase in ammonia being dependent upon the phosphates available for excretion As acido is continues the phosphate excretion falls,

and at this time a glucose tolerance test produces greater rises in blood sugar The fall in plasma bicarbonate is replaced almost molecule for molecule by chloride in Haldane's studies

$CaCl_2$ ingested causes similar change in the plasma bicarbonate and chlorides as shown by Gamble, Ross and Tisdall (1923) The Ca^{++} *is mainly excreted as* $CaCO_3$ *in the feces and the* Cl^- *is absorbed,* replacing HCO_3^- and causing true acidosis with low pH and low $[BHCO_3]$

Both NH_4Cl and $CaCl_2$ are powerful diuretics Haldane produced a fall in weight of 7 pounds in three days after 65 grams of NH_4Cl and a rise in hemoglobin of 20 per cent even although he drank water freely, and Levy noted a similar increase in serum protein after $CaCl_2$ ingestion During NH_4Cl ingestion Haldane observed the following urinary excretions expressed in percentage of the normal values Na 250 per cent, K 520 per cent, Ca 330 per cent, P 180 per cent The diuresis and the salt excretion are attributed by Haldane to the fact that the colloids of the body, being brought nearer their isoelectric point by the fall in pH retain less water and base

The serum Ca has been found by Haldane (1924) to increase 10 per cent following NH_4Cl ingestion $CaCl_2$ ingestion can cause an increase of 25 per cent in serum Ca Sodium bicarbonate conversely *lowers the serum Ca 10 to 20 per cent Ammonium chloride* has been used in the treatment of tetany by Freudenberg and Gyorgy and in lead poisoning by Aub and his associates with striking effect In infantile tetany the symptoms vanish in a few hours and satisfactory results have also been obtained in gastric tetany and post operative tetany In a series of infants treated with 0 5 gram per kilo per day of ammonium chloride the serum calcium rose from a mean value of 6 6 mgm per 100 cc to 8 9, the phosphorus falling from 4 9 mgm per 100 cc to 2 9 Gollwitzer–Meier (1924) has reported a fall in plasma bicarbonate after intravenous ingestion of $MgCl_2$ in rabbits

Excessive introduction of base

Introduction of base into the body increases the bicarbonate of the blood and the pH. Oral bicarbonate administration in human beings has been found by Palmer and Van Slyke (1917) to raise the bicarbonate of the blood according to the assumption that the body contains

70 per cent of fluid and the bicarbonate is distributed throughout this fluid According to this assumption the following equation was calculated Increase in plasma CO in volumes per cent $= \frac{38g}{W}$, g being grams of sodium bicarbonate administered and W the body weight in kilos Or this may be stated that for each mM of bicarbonate administered per kilo, the increment in serum bicarbonate is 1 43 mM This indicates a much wider distribution of the orally administered bicarbonate than of injected HCl or H_3PO_4 in our experiments described above In our experiments with intravenously injected HCl the effect is as if distributed in 1/6 or 1/7 of the body weight instead of in 70 per cent of the body weight The direction of the change in the acid base equilibrium following alkali administration is shown in figure 5

Excessive loss of acid from body

Loss of HCl from the body such as occurs in pyloric obstruction also gives rise to increase in plasma bicarbonate (MacCallum *et al* 1920, Hastings, Murray and Murray, 1921, Grant, 1922), although in the experimental studies of Hastings, Murray and Murray the rise in bicarbonate was not associated with any significant rise in pH

Renal disease

Since the elimination of acid from the body is chiefly by CO elimination through the lungs and by excretion of other acids as acid phosphates and combined with ammonia through the kidneys, it might be expected that impaired renal function would induce acidosis Nephritis and other forms of renal disease associated with impairment of renal function do as a matter of fact give rise to disturbance of acid base equilibrium Wallace and Pellini (1921) point out, however, that double nephrectomy does not produce acidosis in animals and therefore attribute the acidosis of nephritis to some other factor than mere impairment of acid excretion Lepine (1879) and von Jaksch (1888) by titration of the ash of blood demonstrated a diminished alkalinity in the blood of uremics Straub and Schlayer (1912) measured the CO_2 tension of the alveolar air in eight cases of uremia and found in the patient with highest values 34 to 39 mm pCO_2 and in the patient with the lowest, 11 to 17 mm On the basis of this and

the hyperpnea of uremics they suggested that acid intoxication is a factor in uremia Von Hoesslin (1912) pointed out the influence of alkali administration in diminishing the albuminuria and cylindruria in some cases of nephritis and called attention to the difference in amount of alkali necessary to change the reaction of the urine in different cases Sellards (1912) introduced the measurement of the amount of $NaHCO_3$ necessary by mouth to render the urine alkaline as a test for the detection of acidosis and found that whereas 5 grams by mouth suffices in the normal individual, this dose was effective in only one out of nine patients with chronic diffuse nephritis and two patients tolerated 60 and 130 grams respectively intravenously and still excreted acid urine Palmer and Van Slyke (1917) showed that the depletion of the alkali reserve could be better gauged by noting the amount of alkali that must be administered to cause excretion of a *less acid* urine rather than of an *alkaline* urine

Peabody (1914, 1915) showed that as chronic diffuse nephritis increases in severity as measured by impairment of phthalein excretion and retention of non-protein nitrogen in the blood there appears at first little or no evidence of acidosis, then an increase in Sellard's tolerance to alkali and finally in advanced cases, verging on uremia, a diminution in the alveolar CO_2 tension which in the terminal stages of uremia may be very marked He noted there was not a strict parallelism between the other evidences of impairment in renal function and the degree of acidosis but only a general tendency for both to become marked in advanced cases The dyspnea of cardiorenal disease also was commonly more marked than could be accounted for merely by the acidosis Lewis and his coworkers (1913), and Palmer and Henderson (1913) came to substantially these conclusions Chase and Myers (1920) using the method of Van Slyke and Cullen (1917) for the CO_2 capacity of the venous plasma similarly demonstrated this acidosis Marriott and Howland (1916) demonstrated an increase in the phosphate of the serum in nephritis with lowered CO_2 capacity of the plasma and with decreased plasma pH by the dialysis method of Levy, Rowntree and Marriott Greenwald (1915), Feigl (1917a, 1917b), Denis and Minot (1920), Salvesen and Linder (1913) have also shown retention of acid phosphate in nephritics Means and Rogers (1917) reported an extreme acidosis in a man with bilateral polycystic kid-

neys complicated by a septic infection of the hand Two days before death he had extreme hyperpnea with a ventilation of 51 liters per minute, an alveolar pCO_2 (Plesch) of 6 4 mm , a blood urea of 332 mgm per 100 cc (55 mM), a CO_2 capacity of the plasma of 12 vols per cent (5 4 mM), no phthalein excretion in three hours, serum phosphorus of 18 mgm per 100 cc (5 8 mM) serum calcium 3 mgm per 100 cc (0 75 mM) At this time 110 grams $NaHCO_3$ by mouth failed to render the urine alkaline

If one compares the retention of phosphate with the diminution of bicarbonate in the serum it would seem that the acidosis can not always be explained simply by the phosphate retention however In Means and Rogers case with a rise of [P] above the upper normal of 4 3 mM representing about 8 meq of base, there is a fall of $[BHCO_3]$ below the lower normal of 13 mM Some other factor appears to be concerned here than merely phosphate retention The data of Salvesen and Linder (1923) show very little change in the serum base in nephritics beyond the normal limits which makes it probable that acid other than HCO_3^- is increased

MacNider (1920) has demonstrated disturbances in the acid base equilibrium in experimental nephritis in animals and here the acidosis is possibly even more conspicuous than in clinical nephritis This author and also Nagayama (1920) and others have demonstrated improvement in renal function when the disturbance in acid base equilibrium is corrected by alkali therapy and MacNider has demonstrated a diminished susceptibility of the kidney to injury by toxic substances when by alkali therapy the acidosis is prevented

While these studies indicate the depletion of alkali reserve in nephritis, direct studies of the pH of the blood as it exists in vivo such as are furnished by the method of Cullen (1922) are at present available in the literature for only a few cases Cullen and Jonas (1925) cite one case of uremia with plasma pH 6 7 and [CO] of 4 vols per cent Cullen and Stillman at the Rockefeller Institute found a pH of 7 12 with a low normal [CO] of 50 vols per cent in the plasma of a patient with acute convulsions and unconscious but without uremia Strenuous alkali therapy brought the pH to 7 35 and the [CO] to a high normal and the patient recovered from the attack This case is a striking example of the value of pH studies Linder,

Hiller and Van Slyke (1925) report 17 observations on 10 patients with various types of nephritis One case of nephrosclerosis and one of chronic nephrosis showed high normal bicarbonate with normal pH In those forms of glomerulonephritis associated with marked retention of urea there is a tendency to depression of both bicarbonate and pH, the lowest observation being [$BHCO_2$] = 27 8 volumes per cent with pH = 7 16 The zone occupied by these cases is shown in figure 5 Myers and Booher (1924) report 15 cases of nephritis among their 64 cases of abnormal acid base balance, seven of these with less marked depression of [CO_2] showed pH within the lower limit of normal (Hasselbalch's and Van Slyke's compensated acidosis) while eight cases showed abnormally low pH and more marked depression of [CO_2] (uncompensated or true acidosis) However, if the observations on the 15 cases be plotted as in figure 5, the distinction between the compensated and uncompensated groups is seen to be largely an artificial one, due to the width of the normal pH zone The evidence is that any lowering of [CO_2] tends to be associated with more acid pH

Cardiac disease

In cardiac disease uncomplicated by impairment of renal function Peabody (1914) found no evidence of alteration of pH nor of acidosis that could account for the hyperpnea Wilson, Levine and Edgar (1919) found normal CO_2 capacity of the plasma in cases of "irritable heart"

Diabetes mellitus

Diabetes mellitus because of the accumulation of abnormal products of metabolism has long been the classic example of disturbance of acid base balance Of the older literature in regard to presence of acetone bodies there are many reviews The relation of alkali reserve t alveolar CO_2 and to titratable alkali was shown by Stillman, Van Slyke, Cullen and Fitz (1917). It has long been recognized that low alveolar CO_2 tension is an index of low alkali reserve If the low [$BHCO_3$] is accompanied by sufficient decrease in pCO_2 to prevent change in pH, the condition is that of "compensated acidosis" (Hasselbalch, Area 6, Van Slyke) It was also recognized that coma of diabetes is associated with a true acidosis in the sense of decreased pH and decreased [$BHCO_3$]

Before the discovery of insulin diabetic coma was only occasionally strikingly benefitted by alkali treatment

One of the most impressive demonstrations of the action of insulin is afforded by the changes in the acid base balance in diabetic acidosis following insulin treatment Chart No 3, p 547, Cullen and Jonas (1923) shows this most clearly A patient with a plasma pH of 6 98 and [$BHCO_3$] of 16 vols per cent had his plasma restored in one day to a normal range with a plasma pH of 7 32 and [$BHCO_3$] of 41 5 vols per cent The insulin altered the metabolism so that not only did further accumulation of acid bodies cease but the acetone bodies already combined with the base were oxidized thus freeing the base to recombine with CO_2

This chart and figure 5 in the present review also show other interesting facts concerning insulin With excess of insulin the [$BHCO_3$] and pH tend to become abnormally high Such pH studies in stupor from excess of insulin are very few but there are indications in the experiments of Cullen and Jonas that it tends to be associated with an alkalosis (high pH and high CO_2)

One of their patients upon breaking his diet immediately started back toward his initial acidosis but this was easily checked with insulin

In [illegible] data of Cullen and Jonas a tendency to constancy of the pCO rather than of pH is exhibited by a number of individuals, but not in all their cases

These results are in entire agreement with those reported simultaneously by Bock, Field and Adair (1923) and since by Myers and Booher (1924) It would appear that insulin treatment alone, without alkali is sufficient to restore the acid base balance from diabetic acidosis to a normal state or to one of alkalosis It is also evident that the human organism can recover after reduction of the serum pH to as low a figure as 7 0

Rheumatic fever

Because of the old theory that the joint fluid of rheumatic fever was sufficiently acid to liberate free salicylic acid, Boots and Cullen (1922) studied electrometrically and colorimetrically the pH of the joint fluids from such cases Sterile joint fluids showed a pH approximately

that of normal blood In a few of these cases the plasma pH was determined and found to be within normal limits (unpublished results) There is therefore no evidence of a disturbance of the acid base equilibrium in rheumatic fever nor of the possibility of the mechanism of salicylate action mentioned above

Acidosis of children

Howland's and Marriott's (1916) studies indicated that an acidosis is associated with the dehydration of infants Mitchell and Jonas, (1925) found that although low pH and low [CO_2], i e., a true acidosis, might be associated with such conditions, such was not invariably present Further there was no constant relationship between the severity of the disease and the acidosis They conclude that the acidosis when it occurs is a result of the general derangement and not a causative factor in the condition

Fasting

Gamble, Ross and Tisdall (1923) have studied the behavior of the acid base equilibrium during fasting in four epileptic children, with reference to the factors regulating excretion of acid and base. Their studies deal also with the changes in alkali reserve of the serum but do not furnish data concerning the pH

Bigwood (1924b) and Geyelin and Bigwood (in press) have studied the pH and [CO_2] of plasma during the fasting treatment for epilepsy Koehler (1923) found after fasting 50 hours a mean depression of 5 vols per cent [CO_2] and of 0 02 in pH After 77 hours fasting the mean depression in [CO_2] was 8 vols per cent and in pH a fall of 0.10

Anoxemia

The influence of anoxemia in inducing acidosis has been a subject of much theorizing. Wallace and Pellini (1921) studied a variety of toxic substances as to their effect upon the alkali reserve They found that uranium, cantharidin, diphtheria toxin, large doses of arsenic, sodium nitrite when given in a dose large enough to cause methemoglobin formation, and potassium cyanide all produced marked fall in the plasma bicarbonate whereas emetin hydrochloride, hydrazin and

morphin produced no fall of alkali reserve The first four substances mentioned are capillary poisons An extremity poisoned with diphtheria toxin when transfused with normal arterial blood returned the venous blood with a lowered alkali reserve and this led the authors to the belief that the acidosis arose from a disturbance of the muscle metabolism The greater fall in alkali reserve from the first four poisons than following double nephrectomy led them to exclude mere impairment of renal excretion as the important factor in the acidosis Poisons which like emetin and podophyllin are selective for the intestinal capillaries failed to produce any marked acidosis, nor did marked liver injury from hydrazin cause acidosis They concluded that after large doses of sodium nitrite with methemoglobin formation, after cyanide which stops tissue oxidation and after the first four poisons mentioned above, which are general capillary poisons, the acidosis results from interference with tissue oxidation and they emphasize the importance of capillary poisons as a cause of acidosis

This conclusion with regard to cyanide poisoning is confirmed by the work of Holboell (1924), who found that the alkali reserve and pH are reduced in cyanide poisoning and that the venous blood is returned as fully oxygenated as the arterial blood, indicating that the interference with oxygen utilization is in the tissues

pH, lactic acid and ketonuria

Davies, Haldane and Kennaway (1920) have called attention to the fact that when either from hyper-ventilation of the lungs and consequent decrease in the pCO_2, the pH of the blood is increased or when from bicarbonate ingestion the alkali reserve is increased with, or possibly without, increase in the pH of the blood, there is a tendency for acetone bodies to appear in the urine This ketonuria the mechanism of which is not clear, but occurring during an alkalosis, is evidence of the importance of distinguishing between ketonuria and the state of the acid base balance in the blood Macleod and his co-workers (1917, 1918, 1921) have shown that administration of alkali causes an increase of lactic acid in the blood and urine, and an increase of glycolysis in the blood with a decrease in blood sugar concentration Anrep and Canaan (1925) using a Starling heart lung preparation and defibrinated blood found that the lactic acid concen-

tration of the blood rose and fell with the pH of the blood and concluded that the pH of the blood regulates the rate of removal of lactic acid They suggest that this constitutes another factor in addition to the hemoglobin and other proteins and to the bicarbonate and to the phosphates of the blood, in regulating the acid base balance of the blood

The formation of lactic acid during muscle contraction, a complete review of which is given by Hill (1922), has been demonstrated and studied quantitatively especially by Meyerhof and by Hill The extent to which lactic acid can influence the alkali reserve and plasma pH during violent exercise has been shown by Barr and his associates (1923) and by Himwich and Barr (1923) The importance of oxygen in the removal of this lactic acid has also been shown by Meyerhof and by Hill but the extent to which disturbance of this mechanism occurs in disease has not been studied The influence of pH on the recovery process of muscle has been studied by Hartree and Hill (1923, 1924) The direction and possible extent of the disturbance of the acid base equilibrium following exercise is shown in figure 5 from the data of Barr and his associates, and its magnitude is very striking

Anaphylactic shock

It is interesting to consider the evidence for acidosis in anaphylactic shock Eggstein (1921a, 1921b), Underhill and Ringer (1921), Hirsch and Williams (1922), Bigwood, Cogniaux and Collard (1924) and Bigwood (1924a) have demonstrated a fall in alkali reserve in the dog and in man during anaphylactic shock and in the condition of low blood pressure following the injection of protein split products, typhoid vaccines and histamine hydrochloride and Hirsch and Williams and Bigwood and his associates find also a marked fall in pH. These authors have observed some parallelism between the degree of shock or fall in blood pressure and the extent of the acidosis Eggstein found that a fall in the CO_2 capacity of the plasma determined by the technique of Van Slyke and Cullen (1917) to below 25 vols per cent in his experiments was usually associated with a fatal outcome and that a preliminary administration of sodium bicarbonate before the production of anaphylactic shock diminished the acidosis and lessened the mortality Bigwood and his associates point out that the fall in

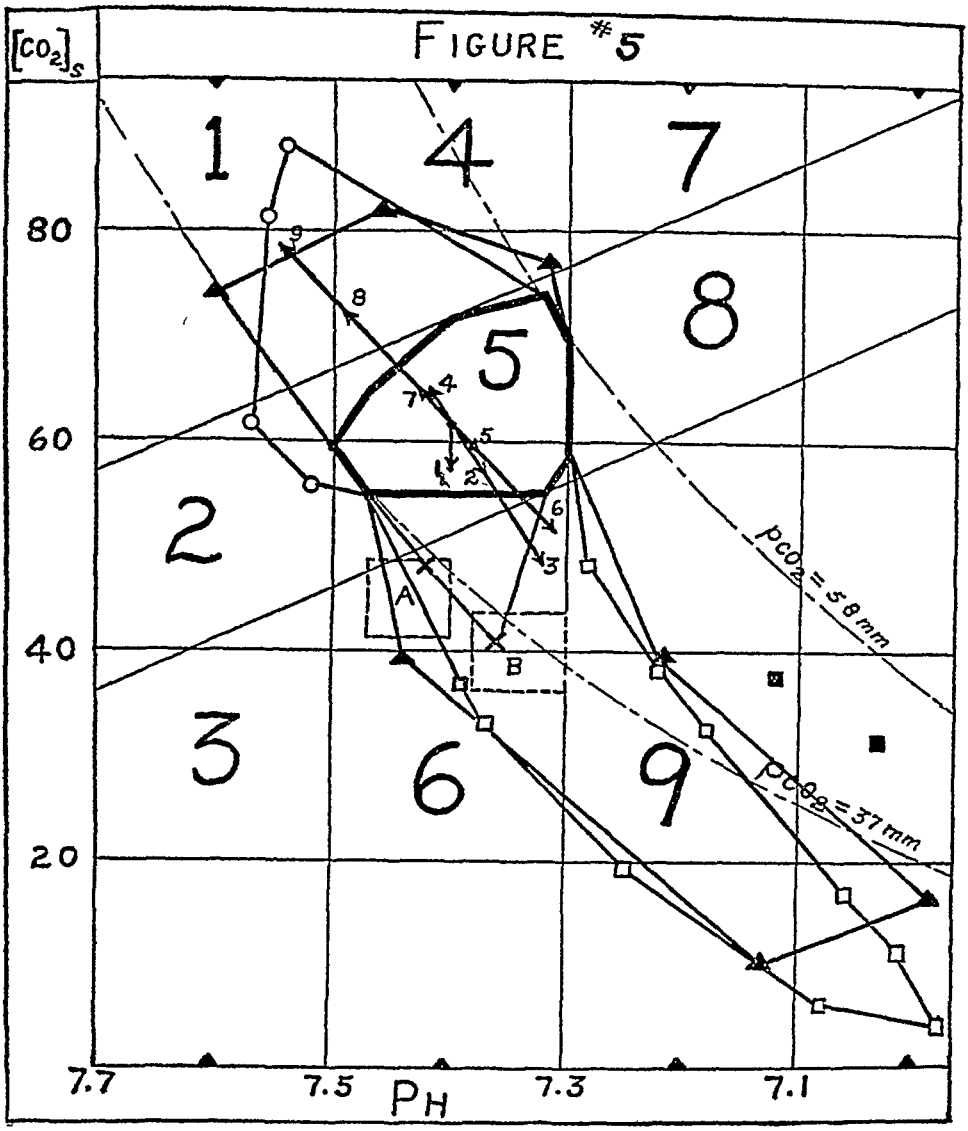
[$BHCO_3$] and in pH involves an increase in the maximal [Ca^{++}] (see our discussion of tetany), a fact possibly of importance in view of Hamburger's evidence for decreased permeability of the capillary wall resulting from increase in [Ca^{++}] The slope of the plotted data of Bigwood and his associates indicates a change in acid base equilibrium quite similar to that which we have observed from anesthesia That is, associated with a true acidosis there is somewhat further depression of pH suggesting a greater depression of the respiratory center than occurs simply from administration of acid, or from nephritic or diabetic acidosis

Traumatic shock

Numerous studies of the alkali reserve in traumatic shock have appeared in the literature which substantially support the findings of Cannon (1918) that there is a lowering of the alkali reserve when there is lowering of blood pressure from shock or from hemorrhage, but his conclusions as to the importance of the acidosis as a cause of the shock have not been established Raymund (1920) has pointed out that the evidences of shock may precede the fall in alkali reserve In five dogs traumatized under local anesthesia no striking fall in the alkali reserve appeared until the condition of the animals became quite serious When general anesthesia is given the effects of the anesthesia upon the acid base equilibrium render the interpretation of the changes due to shock per se very difficult (See later discussion of anesthesia)

Toxic substances

The administration of methyl alcohol to dogs was shown by Pohl to be followed by greatly increased excretion of formic acid in the urine, the maximum excretion being reached on the third or fourth day Krol demonstrated a well defined increase in the ammonia and creatin of the urine in dogs in similar experiments Bongers gave methyl alcohol to dogs and measured the amounts recovered by gastric lavage repeated over several days He asserts that he recovered about three times as much methyl alcohol in the combined washings in the second and third days as he was able to obtain in those of the first, suggesting very slow metabolism of the alcohol In spite of this evidence of abnormal acid excretion Loewy and Munzer (1923b)



VAN SLYKE TYPE OF GRAPH SHOWING NORMAL AREA OF FIGURE 4 WITH ITS LIMITING pCO_2 CURVES AND ITS LIMITING CO_2 ABSORPTION CURVES

Large figures are Van Slyke's areas

1: Uncompensated alkali excess
2, 3: Uncompensated CO_2 deficit
4· Compensated alkali or CO_2 excess
5: Normal balance
6 Compensated alkali or CO_2 deficit
7, 8 Uncompensated CO_2 excess
9. Uncompensated alkali deficit

Numbered arrows indicate direction and magnitude of change induced by

1 Lactic acid (see table 3), 2 NaH_2PO_4 (table 3), 3 HCl (table 3), 4 Ingestion of 84 mgm (1 mM) per kilo of $NaHCO_3$, 5 to 6, immediate effect of one hour of radiation (Kroetz, 1924), 7 to 8, day following radiation (Kroetz), 9 One hour of radiation (Hussey, 1922)

o——o Area including cases of alkali administration or loss of acid by vomiting (Myers and Booher, 1924)

▲——▲ Area including diabetics with acidosis (low[CO_2]) and under insulin treatment (high [CO_2]) (Cullen and Jonas, 1923, Myers and Booher, 1924)

A Fasting 50 hours (Koehler, 1923).

B | Fasting 77 hours (Koehler, 1923)

□— □ Nephritis (Myers and Booher, 1924, Linder, Hiller and Van Slyke, 1925)

■ Effect of exercise (Barr, Himwich and Green, 1923a)

found in rabbits no diminution in the blood bicarbonate Harrop and Benedict (1920) however in a human case of methyl alcohol poisoning found increase of organic acid in the urine and specifically of lactic and formic acids and in addition a fall of plasma bicarbonate to 36 vols per cent Alkali therapy raised the latter to 86 vols per cent pH studies are not available Haskell, Hileman and Gardner (1921) found a reduction in the CO_2 capacity of most of their dogs poisoned with methyl alcohol but not in all, nor was the reduction in CO_2 capacity commensurate with the severity of symptoms Rabinovitch (1922) found a fall in [CO_2] in one human case to 26 vols per cent with a rise in blood phosphorus to 11 2 mgm [P] per 100 cc

It will be seen from figure 5 that the effects of acid or acid producing salts, of fasting, of diabetic acidosis of nephritic acidosis, of severe exercise and probably of anaphylactic shock is to produce change in the acid base equilibrium in a similar direction and to a degree that is dependent upon the severity of the disturbance

Tetany

Tetany is a condition intimately related to the acid base equilibrium of the blood The studies of the pathogenesis of this condition have been recently reviewed by MacCallum (1924) and any extensive repetition of the data there so well presented seems unnecessary Certain aspects of the condition especially in relation to the pH of the serum may be discussed further here to advantage however

It is evident from a study of the literature that tetany is associated with one or more of the following

a Destruction, removal or disease of the parathyroid glands
b Increased alkalinity of the serum
c Diminished [Ca] and increased [P] of the serum

Binger (1917) showed that diminution in the [Ca] and increase of the [P] of the serum produced by injection of phosphate gave rise to marked tetany when the pH of the injected solution was about 6 1 or greater, gave rise to only slight tetany when the pH of the solution injected was 5 8 and to none when its pH was 4 5 With the availability of Cullen's (1922) method for direct measurement of the serum pH the authors in conjunction with H C Gram and H W Robinson carried out

similar experiments thus far unpublished. Dogs were injected intravenously with (a) M/10 Na_2HPO_4, (b) M/10 NaH_2PO_4, (c) M/10 NaH_2PO_4 + M/10 NaCl Each dog received 49 cc per kilo in 53 minutes Blood was taken from the left ventricle before and two minutes after completing the injection, defibrinated under oil and the serum removed without loss of CO_2 Serum analyses are given in table 4

The methods used are the same as those employed in studies of ether anesthesia by Austin, Cullen, Gram and Robinson (1924) [Ca] and [P] were analyzed by the methods of Tisdall (1923) and Tisdall (1922b) respectively.

TABLE 4

SOLUTION INJECTED	TIME OF BLEEDING	pH	[$BHCO_3$]	CALCULATED pCO_2	PROTEIN	[Ca] PER 100 CC	[P] PER 100 CC	[Cl]
			mM	*mm*	*grams per cent*	*mgm*	*mgm*	*mM*
a Na_2HPO_4 $\frac{M}{10}$	Before	7 34	19 3	40	7 1	11 1	2 0	111
	After	7 36	20 4	43	5 4	6 6	27 7	95
b NaH_2PO_4 $\frac{M}{10}$	Before	7 28	18 9	39	8 1	10 8	4 4	111
	After	7 13	9 6	28	6 7	7 0	66 2	99
c NaH_2PO_4 $\frac{M}{10}$, NaCl $\frac{M}{10}$	Before	7 35	20 1	39	6 7	10 4	3 5	111
	After	7 08	11 2	41	4 3	6 3	49 6	113

In the experiments quoted typical tetany appeared in experiment (a), no tetany was present in (b) and (c) It is evident that the greater tendency to tetany in (a) as compared with (b) is not due merely to the larger amount of Na administered in (a) for in (c) where the Na administered is the same no tetany developed The difference in tendency to produce tetany in these as in Binger's experiments lies apparently with the resulting pH of the serum or with the pH and bicarbonate concentration of the serum

Recognition of a relationship of this sort in connection with the physiological activity of Ca together with the recognized limited solubility of Ca led Rona and Takahashi (1913) to attribute the physiological activity of calcium to the calcium ion concentration and,

assuming the blood saturated with $CaCO_3$, to calculate the calcium ion concentration according to the equation

$$[Ca^{++}] = K \frac{[HCO_3^-]}{[H^+]} \tag{20}$$

Brinkman has included the phosphate ions in a similar equation on the assumption that the blood is also saturated with calcium phosphate The most carefully developed form of this double equation is that of Kugelmass and Shohl (1924), who have evaluated the constants for these and related equations in aqueous solutions at 38°C A form of the combined equation given by them is

$$[Ca^{++}] = \sqrt{\frac{(7.6 \times 10^{-6})\ [H^+]}{[HPO_4^{=}]\ [HCO_3^-]}} \tag{21}$$

A fundamental limitation of the usefulness of such an equation has not been brought out however in their discussion Equation (21) is derived by combining the two following equations

$$[Ca^{++}] = 133 \frac{[H^+]}{[HCO_3^-]} \tag{22}$$

which is the maximum $[Ca^{++}]$ possible in an aqueous solution saturated with $CaCO_3$, and

$$[Ca^{++}] = \frac{(67 \times 10^{-6})}{[HPO_4^{=}]} \tag{23}$$

which is the maximum $[Ca^{++}]$ possible in an aqueous solution saturated with $CaHPO_4$ In discussing the use of a combined equation such as (21) it has commonly been overlooked that such an equation is valid only when the system is saturated simultaneously with both $CaCO_3$ and $CaHPO_4$ and furthermore that under these conditions the following conditions are obligatory

1 A fixed ratio must exist for $\frac{[HCO_3^-]}{[HPO_4^{=}]}$

2 The $[Ca^{++}]$ calculated from equation (22) will equal the $[Ca^{++}]$ calculated from equation (23) and also of course that calculated from equation (21)

Whenever the $[Ca^{++}]$ calculated from equation (22) differs from that calculated from equation (23) then the system is saturated only with

the salt corresponding to the equation giving the lower calculated [Ca^{++}], and that value represents the maximum possible [Ca^{++}] in the system Under these conditions, equation (21) is no longer valid for it is based on the assumption that the system is saturated with both carbonate and phosphate. The proper use for the equations in so far as the results obtained on pure aqueous solutions of the salts can be applied to serum, would be to use equations (22) and (23) separately and take as the significant figure the lower calculated value as representing the maximum possible calcium ion concentration for the serum This method we applied to the experimental data given in table 4 with the results shown in table 5. The values in parentheses can have, as pointed out, no real significance With the assumptions that we are making here, the maximal calcium ion concentration after

TABLE 5

	EXPERIMENT	[Ca^{++}] EQUATION (22) (CARBONATE)	[Ca^{++}] EQUATION (23) (PHOSPHATE)	TETANY
a	Before ..	0 41	(1 24)	
	After	(0 37)	0 09	Marked
b	Before.	0 37	(0 58)	
	After ..	(1 03)	0 04	None
c	Before .	0 30	(0 71)	
	After . .	(0 99)	0 06	None

injection is in each instance limited by the concentration of $HPO_4^{=}$ and there is no relation between this maximal [Ca^{++}] and the presence or absence of tetany.

It would seem therefore that the influence of pH upon the development of tetany cannot be accounted for merely by its influence upon the maximal possible calcium ion concentration. It seems probable that the pH has an additional physiological effect in influencing the susceptibility of the organism to the calcium ion concentration or to the balance of the inorganic ions.

Hemorrhage

Wilson (1923) has pointed out that the immediate effect of a large experimental hemorrhage is fall in blood bicarbonate, soon

followed, however, by a rise above normal associated with an increase of pH above normal

Gastric disorders

In disease of the gastro intestinal tract the only striking alteration observed in acid-base equilibrium is the alkalosis associated with persistent vomiting and consequent loss of acid from the body in the form of HCl This alkalosis may give rise to tetany MacCallum and others (1920) in experimental pyloric obstruction obtained relief of the tetany with injections of NaCl

MacAdam and Gordon (1922) have reported the finding of an alkalosis in cases of periodic vomiting associated with definite ketonuria This constitutes another instance of the danger of assuming a condition of acidosis to be present because acetone and diacetic

TABLE 6

	AVERAGE pH
12 normals	7 29
8 miscellaneous diseases	7 33
23 carcinoma cases	7 34

acid are present in the urine Following prolonged alkaline treatment for gastric or duodenal ulcer, Hardt and Rivers (1923) have reported toxic manifestations associated with rise in the plasma [CO] and with evidences of impairment of renal function

Myers and Booher (1924) report several cases of high alkali both compensated and with high pH following Sippy treatment for ulcer They also write "we are inclined to think that alkalosis is a condition overlooked and sometimes confused with acidosis by the clinician We believe great care should be exercised in administration of alkali"

Neoplasms

In cases of carcinoma the serum ash was found by Moore and Wilson (1906) to require slightly more acid for its neutralization than normal serum and this observation was confirmed by Watson (1909) Menten (1917) reported an increase in the pH of serum of carcinoma cases, but later studies by Chambers and Kleinschmidt (1923) in which the pH was calculated from the CO_2 absorption curves and the CO_2 content

of the blood as drawn with correction for oxygen unsaturation shows the averages given in table 6

Myers and Booher (1924) in a series of 11 cases of uncomplicated carcinoma found no change in the acid base equilibrium, nor did Corran and Lewis (1924)

It seems probable therefore that there is no significant change in the serum pH in carcinoma

Radiation

The effect of x-ray radiation has been studied in rabbits by Hussey (1922). He observed an increase in both plasma CO_2 capacity and pH by Cullen's method, evident one hour after radiation and still present after 48 hours The increase in pH in three hours was from 0 11 to 0 18 and in the plasma CO_2 capacity from 16 to 18 vols per cent

Kroetz (1924) has found in man after both x-ray and ultraviolet radiation a fall during the first hour of 3 to 10 vols per cent in the serum bicarbonate and of 0 02 to 0 09 in pH, followed by a rise of both [$BHCO_3$] and pH the following day to about as much above the initial value These high values may persist for a few days These changes are consistent in all of his observations, but are obviously small

Balderrey and Barkus (1924) have observed the effect of exposure to sunlight upon the pH of patients as measured by Cullen's (1922) method. They found no effect on cloudy days but on bright days an increase in the average pH of 0 17 When pigmentation was marked they observed less change in pH

Reference to figure 5 will show that the blood after administration of alkali and at a certain stage after radiation with ultraviolet light or x-ray is characterized by a change in acid base equilibrium opposite to that produced by administration of acid both as regards [$BHCO_3$] and pH. On the other hand, the rise in bicarbonate seen in certain diabetics treated with insulin shows a tendency to be associated with normal rather than with increased pH

Surgical anesthesia

In surgical anesthesia we have evidence of a combination of acidosis in the sense we are using the term and of depressed ventilation

In discussions and studies of acidosis following general anesthesia with or without operation, the evidences of abnormality which have been taken as evidence of acidosis may be considered in three groups

1 Changes in plasma bicarbonate and pH
2 Ketonuria
3 Certain clinical symptoms

Diminution in the plasma CO_2 during and immediately after ether anesthesia in man and animals has been observed by Austin and Jonas (1917) Caldwell and Cleveland (1917), Carter (1920), Collip (1920), Van Slyke, Cullen and Austin (1922), Leake, Leake, and Koehler (1923), and Austin, Cullen, Gram and Robinson (1924)

The fall of plasma CO_2 in man during operation under general anesthesia has been found to be from about 4 to 10 vols per cent with return to approximately normal within twenty four hours Various types of anesthesia differ only in a minor degree in the amount of fall produced and even in operations under local anesthesia Caldwell and Cleveland observed some fall Such changes in the alkali reserve are relatively small In dogs under ether anesthesia the fall may be occasionally considerably greater, amounting in one instance to 22 vols per cent (Van Slyke, Cullen and Austin (1922)), but as a rule in dogs also the fall is less than 10 vols per cent

The change in pH has been less extensively studied Menten and Crile (1915) reported a fall in the blood pH in rabbits and Van Slyke Cullen and Austin (1922) demonstrated fall in pH in the dog under both light and deep ether anesthesia Leake, Leake and Koehler (1923) confirm the finding Cullen, Austin, Kornblum and Robinson (1923) have shown that this fall occurs chiefly during the early minutes of anesthesia and Austin, Cullen, Gram and Robinson (1924) have demonstrated that it is due chiefly to the introduction of some unidentified acid into the blood, together with Cl^- anions The possibility that the unidentified acid is lactic acid has not been excluded That lactic acid formation in the muscles is actually largely responsible for the fall in alkali reserve in the dog under ether anesthesia has now been demonstrated by Ronzoni, Koechig and Laton and they have also discussed a possible relation of this with the observations of Stehle and Bourne (1924)

Stehle and Bourne (1924) have shown following ether anesthesia an increased phosphorus elimination in the urine, a 2 5 per cent decrease in muscle phosphorus and a 12 5 per cent increase in liver phosphorus The change in blood phosphorus, however, is slight which taken into consideration with the base findings of Austin, Cullen, Gram and Robinson (1924) indicates that the explanation of the diminished alkali reserve is not a withdrawal of base from the blood consequent upon mobilization of muscle phosphoric acid On the other hand the view advanced by Y. Henderson and Haggard (1918) that the fall in alkali reserve was a compensatory phenomenon consequent upon hyperpnea and excessive removal of CO_2 in the early stages of anesthesia has not been supported by the other studies mentioned

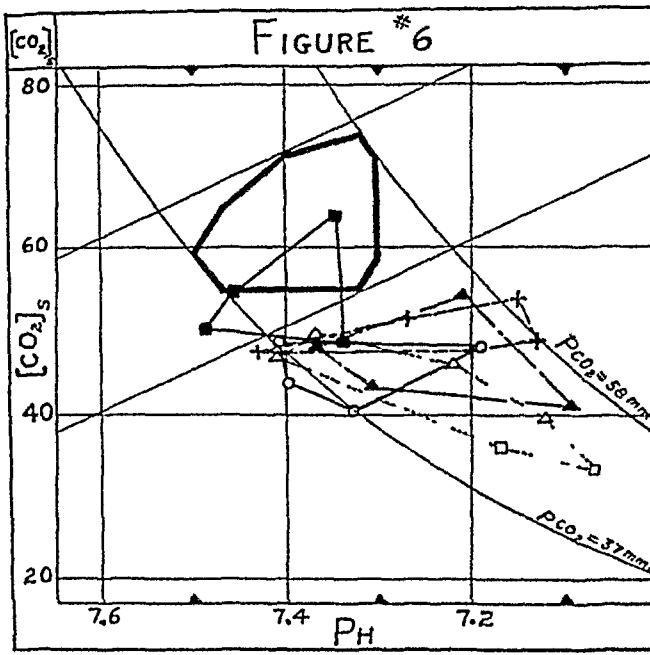
Koehler (1924) has studied the acidosis of anesthesia as it occurs in human subjects The changes observed are comparable to those found in experimental studies Recovery of the acid base balance is fairly rapid and usually complete in from one to three hours Koehler divides the recovery into two phases that from excessive pCO_2 which is rapid occurring within the first hours and that from depressed alkali reserve which is slower

Leake and Hertzman (1924) have studied the effects of ethylene-oxygen and nitrous oxide-oxygen anesthesia on the acid base equilibrium. They observed changes in the acid base equilibrium similar to but less marked than those observed with ether and chloroform In addition, the factor of anoxemia is of great importance in these types of anesthesia. There may be an initial increase in pH if the anoxemia is marked Leake (1924) has also discussed the disturbances of carbohydrate metabolism following ether anesthesia

The studies of White (1923) upon the beneficial effect of post-operative administration of CO_2 as a respiratory stimulant to accelerate the removal of ether through the lungs, a procedure which in itself necessarily tends for the time toward still further lowering the pH, indicate that the fall in pH is of less importance than the other effects of the ether The procedure adopted by White led to a diminution of disagreeable post-operative symptoms

That ketonuria as shown by qualitative tests for acetone and diacetic acid is a common finding after surgical anesthesia has long been noted and has been taken as evidence of acidosis Reimann

and Bloom (1918) and Caldwell and Cleveland (1917) demonstrated that ketonuria could often be detected immediately before operation,



VAN SLYKE TYPE OF GRAPH SHOWING NORMAL AREA OF FIGURE 4 AND EFFECTS OF ANESTHESIA

(From data of Cullen, Austin, Kornblum and Robinson (1923) in dogs and of Foehler (1924) in human cases.)

■——■ Before anesthesia
+——+ Human cases under nitrous oxide
▲——▲ Human cases under ether
○——○ Human cases after nitrous oxide
△——△ Human cases after ether anesthesia
□——□ Dogs after ether anesthesia

probably due to the preliminary fasting, but that it was much more frequent following the operation. On the other hand, they established the fact that there was no constant relation between the severity or

duration of the operation or the seriousness of the post-operative course, or the degree of reducton in the alkali reserve on the one hand, and the degree of the ketonuria on the other Indeed, it seems possible that the ketonuria is mainly an expression of the fasting incident to operation, and there is no evidence that it is of any serious clinical importance

Of interest in this connection, however, are the observations of Thalhimer (1923), of Fisher and Snell (1924) and of Ginsberg (1924) upon the effect of insulin and carbohydrate given after operation, with a reported disappearance of ketonuria and lessening of disagreeable post-operative symptoms The experimental studies of Stewart and Rogoff (1917) and of Ross and Davis (1920) are of especial interest in this connection.

A third group of phenomena described following operation and sometimes attributed to acidosis, probably chiefly because the common ketonuria has been examined for and noted in these cases, is a clinical picture which is essentially that of post-operative shock The evidence that these cases actually suffer from acidosis is however lacking These cases are not numerous in the surgical clinics and the opportunities for accurate studies of the blood are still rarer In two such cases studied by the authors and believed by the surgeons to be post-operative acidosis, both showed plasma [CO_2] value above normal.

It seems probable therefore that grouped under the name of acidosis three more or less distinct pathological conditions have been recognized as occurring at times after general anesthesia with or without operation Of these, the one of chief clinical importance is that characterized above as a form of post-operative shock, but this condition is probably often not accompanied by acidosis and is probably not due to acidosis

The ketonuria so often observed after operation probably arises in many instances from the fasting preceding and following operation In itself it is of doubtful clinical importance It may be associated with alkalosis rather than with acidosis The value of treatment with carbohydrate with or without insulin must be based upon the effect of such treatment on the post-operative symptoms

The fall of plasma bicarbonate and of pH, which alone should be

called acidosis, appears to be a constant accompaniment of experimental and surgical ether, chloroform and nitrous oxide anesthesia, but there is no satisfactory evidence that it is of clinical importance in human surgery When the disturbances of the acid base equilibrium that occur normally with vigorous exercise, as shown by the studies of Barr and his associates (1923) are called to mind, it may well be questioned whether the exertion incident to passing under the anesthetic may not be an important factor in this acidosis exaggerated possibly by interference under the anesthetic with normal tissue oxidation It is not at all clear that the acidosis is a menace to the individual or that alkali therapy is indicated With removal of the anesthetic spontaneous restoration of the acid base equilibrium is rapid as is the case after vigorous exercise Failure of the acid-base equilibrium to recover after operation occurs probably only incident to some serious disturbance of the metabolism, and is not so far as we know a consequence of the acidosis per se

Lobar pneumonia

In certain infectious diseases various workers have reported disturbances of the acid base equilibrium The most thoroughly studied infection is lobar pneumonia

Palmer (1917) demonstrated the excretion in the urine in lobar pneumonia of large amounts of organic acid which at a pH of 5 0 was present as free acid At this pH, 87 per cent of uric acid is free, one third of β hydroxybutyric and of acetic but only about 5 to 7 per cent of hippuric, diacetic and lactic acids This fact excludes the last three from consideration as the acid excreted in pneumonia Palmer excluded by analysis β hydroxybutyric acid, uric acid and ethereal sulphates The nature of the acid was not further identified There may be a relation between these observations and those of Barach, Means and Woodwell (1922) who observed a tendency to lowering of the alkali reserve in pneumonia They found in 10 cases an average CO_2 capacity in arterial blood at 40 mm pCO_2 at 37° of 43 2 vols per cent as compared with the average for normal individuals found by Peters, Barr and Rule (1921) of 49 3 vols per cent, the lowest observed in pneumonia was 35 0 vols per cent Barach, Means and Woodwell calculated the arterial pH from the CO_2 absorption curve and obtained

an average in their 10 cases of pneumonia of 7 31, with four cases below 7 30 which they take as the lower limit of normal resting pH The lowest pH they observed was 7 20 They found no relation, however, between the pH or the reduction in the alkali reserve and the prognosis or the degree of anoxemia They observed a spontaneous rise in the alkali reserve and return of pH to normal at, or shortly after, crisis in three patients studied before and after crisis In another patient they observed the same restoration without crisis after vigorous oxygen therapy. Binger, Hastings and Neill (1923) have reported edema occurring during convalescence from pneumonia in association with bicarbonate administration Hastings, Neill, Morgan and Binger (1924) have more recently studied a series of 30 pneumonia patients, making direct determination of the pH and [CO_2] They observed a lower arterial CO_2 tension during the febrile period than after return to normal temperature in seven cases but lowered pCO_2 and increased oxygen unsaturation did not occur together with sufficient regularity to indicate a causal relationship They noted no tendency towards an acidosis of either metabolic or respiratory origin The alkali reserve was within or near normal limits in every case and the pH was in each instance within normal limits, 7 30 to 7 50, and in most instances in the more alkaline half of this range Their results contraindicated alkali therapy in all the cases studied In 8 of the 10 cases in which arterial oxygen saturation was determined an abnormally low saturation was observed at some stage of the disease Taken with the non-occurrence of increased CO_2 tension these results they concluded support the view that when the mechanism for gas exchange in the lungs is affected, absorption of oxygen fails before elimination of carbon dioxide is significantly impaired.

Disturbances similar to those produced by hypopnea or hyperpnea

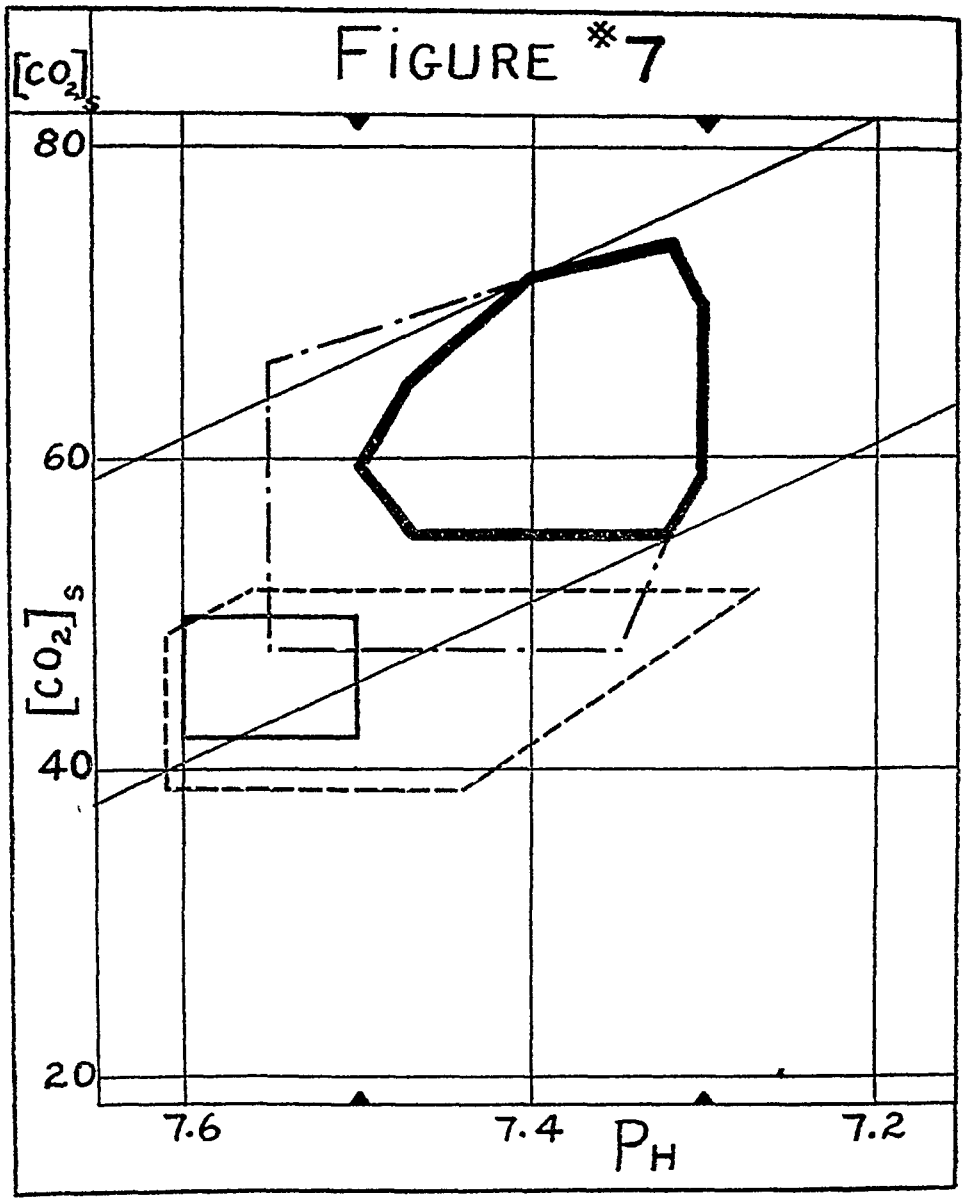
Disturbances in the excretion of CO_2 either by a hyperpnea leading to a lowering of the alveolar pCO_2 or by impaired pulmonary ventilation with rise of pCO_2 produce changes in the blood that have been extensively studied The subject has been thoroughly and extensively reviewed by Van Slyke (1921a, 1921b) with regard to the mechanism by which the pH of the blood is related to its pCO_2, and a discussion here of the development and present knowledge of this phase of the

subject would therefore be superfluous The relation of changes in pH of the blood to heart, bloodvessels and the centers of the central nervous system has been touched upon already

In the study of disease so long as we were limited to the measurement of the plasma or blood [CO_2] and to the direct measurement of the alveolar pCO_2 with its well recognized difficulties in untrained or very ill patients the recognition of disturbances in acid base equilibrium consisting chiefly of hypoventilation or of hyperventilation was difficult With the increasing data on both plasma or blood [CO_2] and direct measurement of pH of the serum there appears to be emerging evidence of disturbances of this type in certain diseases

Infections

Hachen and Isaacs (1920) and Koehler (1923) have found in influenza, influenzal bronchopneumonia and grippe with temperatures above 103° and the latter in one case of peritonitis a reduction in the CO_2 capacity of the plasma and CO_2 content of the blood which may be rapid or gradual in its development Koehler found the pH of the venous blood, however, increased rather than diminished and believes the fall in blood bicarbonate may be at least in part compensatory to a febrile hyperpnea, analogous to the shift in acid base equilibrium which Bazett and Haldane (1921) observed on immersion in a hot bath and which Koehler (1923) confirmed The latter's observations are plotted in figure 7 Yamakita (1921) however observed a fall in alkali reserve in experimental infections even when the temperature was not very high but only with very great increase in temperature in hyperthermia due to heat puncture Hirsch and Williams (1922) found in rabbits infected by intravenous injection of pathogenic bacteria a marked lowering of both CO_2 capacity of the plasma and of pH and Leake, Vickers and Brown (1921) observed similar changes in experimental B bronchisepticus pneumonia in dogs The association of the fall in [$BHCO_3$] in experimental pneumonia and other experimental infections with a lowered pH suggests that febrile hyperpnea is not always the important factor in the shift of acid base condition but that there may be depletion of the alkali reserve in certain infections The possibility of a renal factor is to be considered in these infections



VAN SLYKE TYPE OF GRAPH

Normal area of figure 4

Area of febrile cases (Koehler, 1923)

Area of individuals in hot baths (Koehler, 1923)

Area of individuals in last sixteen weeks of pregnancy (Marrack and Bo[illegible])

areas lie almost wholly within the normal CO_2 absorption curves but at [illegible] (Van Slyke's "uncompensated CO_2 deficit")

Reports of ketonuria in the course of infections cannot be taken as evidence of acidosis Ketonuria indicates a disturbance of the normal fat metabolism In diabetes mellitus this is constantly associated with acidosis Ketonuria, however, can be produced by administration of alkali as already pointed out and in the discussion of the effects of ether anesthesia we have pointed out that ketonuria is not proportional to the acidosis present

Pregnancy

The association of toxemias of pregnancy with increased NH_3/N in the urine has long been noted Losee and Van Slyke (1917) showed however that in pregnant patients with pernicious vomiting and strikingly high ammonia figures the plasma bicarbonate may indicate no greater degree of acidosis than may be observed in non-toxic pregnancy Sellards (1912) reported a similar case with only a normal tolerance for sodium bicarbonate Losee and Van Slyke, (1917) Williamson (1923), Cook and Osman (1923), and Marrack and Boone (1923) have shown some diminution of the bicarbonate of the blood in normal pregnancy The latter authors in seventeen cases in the last sixteen weeks of pregnancy found bicarbonate of 47 to 65 vols per cent as compared with 58 to 65 vols per cent for normals Cook and Osman found the serum bicarbonate in pregnant women to be on the average 85 per cent of that for normal non-pregnant women Hasselbalch and Gammeltoft (1915) noted a greater lowering in alveolar pCO in the later months of pregnancy than in the bicarbonate, and Marrack and Boone observed the same Consistent with this is the observation by Marrack and Boone of a pH of 7 35 to 7 55 by Cullen's method (1922) in their pregnant cases, as compared with 7 30 to 7 45 for normal cases (see fig 7) This would indicate a tendency to increased pulmonary ventilation in late pregnancy, and the slight lowering of the [BHCO$_3$] may well be considered therefore a secondary effect of hyperventilation rather than as due to true acidosis

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ANOXEMIA IN LOBAR PNEUMONIA

Its Causes, Pathogenesis, and Significance

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I. THE OCCURRENCE OF ANOXEMIA IN LOBAR PNEUMONIA. THE GENERAL PHYSIOLOGICAL CAUSES AND RESULTS OF ANOXEMIA

Acute lobar pneumonia became more than one hundred years ago recognized as a nosologic morbid entity through investigators belonging to the so-called anatomic school. Our conception of this disease passed unchanged even the bacteriologic era during which a variety of morbid entities mainly built on their symptomatology became split and collected into new entities. In spite of the fact that there are but few diseases which in respect to symptomatology, diagnosis, and treatment are more familiar to the clinician than lobar pneumonia, it is true that no other respiratory disease has during the last few years given rise to more varying, more scientifically fundamental, and more practically important problems.

The recent progress in the study of the etiology and epidemiology of lobar pneumonia and the specific and prophylactic treatment based upon these studies will not be entered upon in spite of the fact that the most important problems concerning lobar pneumonia are of etiologic and epidemiologic nature. We shall confine ourselves to a discussion of the pathological physiology of the disease, where we face questions which scientifically and practically are of almost as much

[1] The public competition for the chair of Internal Medicine at the University of Copenhagen held in September, 1923, required of each candidate four lectures, two to be given on two successive days on a subject chosen by the candidate, one a clinical lecture on a patient assigned by the committee the day before, and one on a subject also assigned the day before and common for all the candidates. The two lectures given here were chosen by the candidate. In the translation from Danish the original form is preserved as completely as possible.

importance as the etiological problems As long as lobar pneumonia exists—and there is no reason to believe that this disease belongs to the exterminable group of infectious diseases—it is important to study the degree of the functional disturbances caused by the infection Because, if a functional disturbance affects an organ, the functional power of which cannot be reduced beyond certain limits without serious danger for the body, this disturbance may be of vital importance for the course of the disease Treatment of such functional disturbance may, therefore,—although of symptomatic nature—prove to be not less important than the specific and prophylactic treatment

By far the most important functional disturbances, which occur in patients suffering from lobar pneumonia, concern respiration and circulation In this paper certain disturbances in the function of the lungs will be discussed with special reference to the nature and cause of these disturbances and to their significance for the body as a whole The main respiratory functions of the lungs—intake of oxygen and output of carbon dioxide—are often disturbed in patients with lobar pneumonia We shall in this lecture confine ourselves to a discussion of disturbances in the oxygen intake and discuss questions concerning the carbon dioxide only in case they are of importance for the oxygen intake.

Oxygen content of arterial blood in patients with lobar pneumonia

Investigations published within the last few years have shown that the arterial blood, in a large percentage of cases, contains less oxygen in proportion to the hemoglobin than does arterial blood from normal individuals Or, in other words, the arterial saturation is abnormally small in a number of patients with lobar pneumonia

Table 1 gives the percentage of oxygen saturation of the arterial blood in 50 patients, compiled from eight different publications In 12 instances the degree of saturation was found to be between 90 and 100 per cent, which is either normal, or at most, only slightly diminished In 22 instances the degree of saturation fell between 80 and 90 per cent, in 9 instances between 70 and 80 per cent, and in 7 instances between 60 and 70 per cent

TABLE 1

Oxygen saturation of the arterial blood in 50 patients with lobar pneumonia and in 14 patients with influenzal pneumonia

AUTHOR	YEAR	CLINIC	NUMBER OF PATIENTS EXAMINED	OXYGEN SATURATION OF ARTERIAL BLOOD					REMARKS
				100–90 per cent	90–80 per cent	80–70 per cent	70–60 per cent	< 60 per cent	
Hutter	1912	Marburg University	2		1(1)*	1			
Stadie, I	1919	Rockefeller Hospital	6	2	2	2(1)			
Meakins	1921	Edinburgh University	10	2	8				Oxygen treatment
Barach and Woodwell	1921	Harvard University	6	2	2	2			Oxygen treatment
Stadie, II	1922	Rockefeller Hospital	5			2(1)	3		Oxygen treatment
Le Blanc	1922	Eppendorf Hospital	6	1	4(1)	1(1)			
[illegible]	1923	Rockefeller Hospital	1				1		Oxygen treatment
[illegible]	1923	Rockefeller Hospital	14	5(1)	5(3)	1(1)	3		Oxygen treatment
Total			0	12	22	9	7		
Mortality			11	1	5	5	0		
Stadie, I: Influenzal pneumonia	1919	Rockefeller Hospital	26	6	7	7	2	4	
	Mortality		14	0	2	7	1	4	

* Figures in parentheses indicate dead

The various columns in figure 1 correspond to the average values for the different groups given in table 1 The light part of the column indicates the oxyhemoglobin, whereas the dark part represents the reduced hemoglobin

The degree of arterial oxygen saturation has been shown to vary considerably during the course of the disease So far the determinations are too few to justify an attempt to draw parallels between the degree of oxygen saturation and the various clinical and anatomical stages of the disease

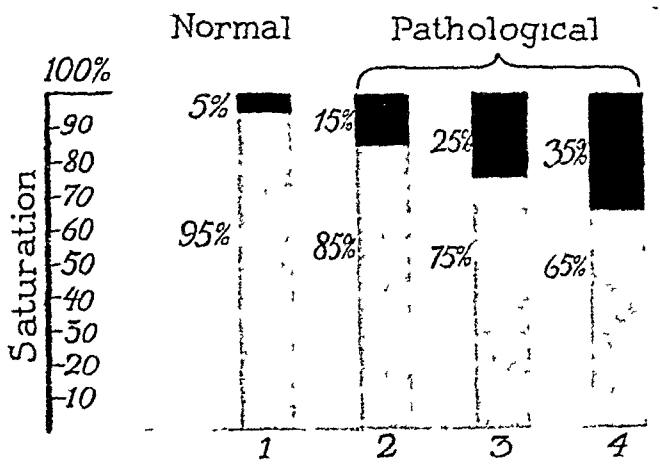

FIG 1 DIAGRAM OF THE ARTERIAL OXYGEN SATURATION OF THE VARIOUS GROUPS OF PATIENTS IN TABLE 1

Dark areas indicate reduced hemoglobin Light areas indicate oxy-hemoglobin

In table 1 below is given the arterial oxygen saturation in a series of patients with influenzal-pneumonia where—as we shall briefly discuss later—a decreased arterial oxygen saturation is still more commonly seen than in patients with lobar pneumonia

The cause of the diminished oxygen content of the arterial blood in lobar pneumonia

In order to facilitate a discussion of the cause of the diminished arterial oxygen saturation in pneumonia, it might be useful first to call attention to the three diagrams in figure 2, where the ratios of

reduced hemoglobin to oxyhemoglobin in the various parts of the circulatory bed are given

Diagram *A* represents the conditions found in normal individuals at rest The blood enters the arteries normally saturated with oxygen (about 95 per cent) In the tissue capillaries about one-fourth of the oxygen goes out to the tissues so that the venous blood becomes only three-fourths saturated, which with an oxygen capacity of 20 volumes per cent corresponds to an oxygen content of 15 volumes per cent Diagrams *B* and *C* indicate the two ways in which the arterial

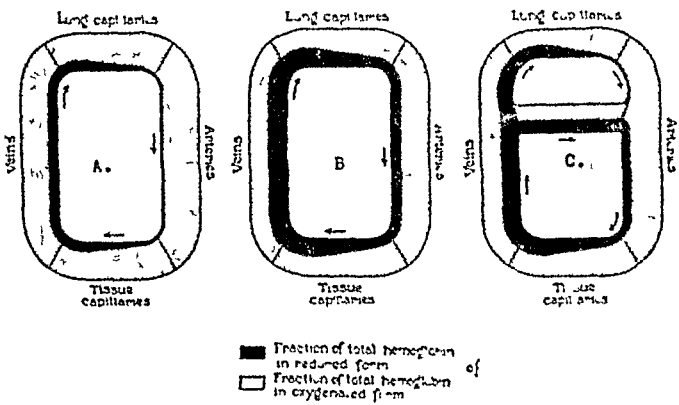

FIG 2 DIAGRAMS SHOWING THE PROPORTION OF OXYGENATED HEMOGLOBIN TO REDUCED HEMOGLOBIN IN DIFFERENT PARTS OF THE CIRCULATORY SYSTEM

A, in normal individuals, *B*, in a case of incomplete oxygenation in aerated parts of the lungs, *C*, in a case where a fraction of blood passes through completely unaerated parts of the lungs

oxygen content may be diminished by pathological lung conditions In *B* all the blood is oxygenated, but to a lower degree than usual, because less oxygen for one or another reason passes from the alveoli to the blood streaming through the lung capillaries In *C* the arterial oxygen saturation decreases because a fraction of the blood passes through a part of the lung to which air has no access

It might seem most natural to presume that the cause of the low arterial oxygen saturation so often found in lobar pneumonia is that a

fraction of the blood passes the consolidated area to which air has no access (diagram *C*) Various facts can, however, hardly be brought in accordance with such a conception First, the dry bloodless condition of the consolidated parts of the lungs, particularly in the stage of gray hepatization, points against any considerable perfusion of the consolidated area Secondly, as Kline and Winternitz, and later Gross showed, only the largest branches of the *arteria pulmonalis* could be injected with an emulsion of either Berlin blue or barium gelatine Third, extensive consolidation of the lung tissue is found without any decrease in the oxygen content of the arterial blood

TABLE 2

Comparison between number of lobes consolidated and degree of arterial oxygen saturation in 14 patients with lobar pneumonia (Rockefeller Hospital)

NUMBER OF LOBES CONSOLIDATED	ARTERIAL OXYGEN SATURATION, PER CENT
1	82 1–84 0–90 2–91 6–97 2*
2	68 0–75 8–86 5–94 4–97 0
3	64 6–68 2*–90 5
4	92 7

* Indicates that the patient was treated in oxygen chamber when the arterial blood was drawn

In table 2 is given the degree of arterial oxygen saturation in per cent of capacity and the number of affected lobes in a series of patients from the Rockefeller Hospital examined by Hastings, Neill, Morgan and Binger Besides the routine stethoscopical examinations, roentgenograms were made in all instances No parallelism between degrees of arterial oxygen saturation and extent of consolidation is seen in these patients Fourth, in a number of cases it has been possible by oxygen therapy completely to oxygenate the abnormally unsaturated arterial blood In such cases it can be excluded that any blood has passed a part of the lung to which air has no access (anatomical shunt). The decreased arterial oxygen saturation found in pneumonia must therefore at least in a certain number of cases be explained in another way

English investigators especially Haldane, Meakins and Priestly, consider shallow breathing mainly responsible for the decreased arterial oxygen saturation When the depth of respiration becomes so small

that only the dead space, which is about 150 cc., is ventilated, the air in some or all alveoli must be renewed by diffusion alone The normal alveolar oxygen tension is not maintained and if the oxygen tension decreases sufficiently, incomplete saturation of the blood passing through the lung capillaries may result Figure 2, *B*, indicates such a condition This theory cannot be said to be absolutely new in principle, because a similar explanation has been given for the dyspnoea of certain heart patients However, the English investigators have not only gone deeper into the problems than before, they have also produced experimental support for a theory which previously had been a mere hypothesis In the first place it has been shown by these three investigators that superficial lung ventilation, artificially produced in normal subjects by means of the so called concertina, would cause periodic respiration, which is known to be due to oxygen want Secondly, Meakins and Davies have shown that shallow respiration may cause decreased arterial oxygen saturation in normals They found, for instance, that the arterial blood was 94 3 per cent saturated during normal respiration If the tidal air was diminished from the normal value of 500 cc to 315 cc the arterial oxygen saturation decreased to 91 7 per cent Further decrease in the tidal air caused periodic respiration Thirdly, Meakins has determined the depth of respiration and at the same time observed the skin color in 4 patients with lobar pneumonia

He found that the color became cyanotic if the depth decreased to about 250 cc (table 3) Binger, Hastings, and Neill observed the depth of respiration, the arterial unsaturation and the skin color in a patient with lobar pneumonia They found, as shown in table 4 that the arterial oxygen saturation rose and the cyanosis disappeared on increasing the depth of respiration On the other hand it does not seem likely that shallow breathing is the only cause responsible for the decreased arterial oxygen saturation in patients with pneumonia It is beyond doubt that cyanosis of respiratory origin may occur in patients where the depth of respiration is not below normal Stadie in a discussion of this problem, puts very little stress on shallow breathing as the cause of low arterial oxygen in pneumonia It can furthermore not be excluded *a priori* that rapid and shallow breathing may be the result and not the cause of low arterial oxygen in pneumonia

At any rate, another explanation than shallow breathing, must be found for the low arterial oxygen in a certain number of patients with pneumonia It is not unlikely that the cause is decreased diffusion from the alveolar air of normal composition to the blood in the lung capillaries Such a diminished diffusion may be brought about in two ways first, by the presence of a layer of fluid on the inside of the alveoli, as has been suggested by Hoover as the cause of the cyanosis

TABLE 3

Relation between depth of respiration and skin color in 4 patients with lobar pneumonia (Meakins)

NUMBER OF PATIENT	DEPTH OF RESPIRATION	CYANOSIS
	cc	
1	270	0
	230	+
2	290	[illegible]
3	270	0
	240	+
4	260	0
	230	+

TABLE 4

Relation of arterial oxygen saturation and skin color to depth of respiration in a patient with lobar pneumonia (Binger, Hastings and Neill)

DEPTH OF RESPIRATION	ARTERIAL OXYGEN RESPIRATION	CYANOSIS
cc	*per cent*	
176	61 0	+
450	91 7	0
540	96 0	0

in bronchopneumonia, and by Barcroft as the cause of the cyanosis in cases of gas poisoning during the war Second, such a diminished diffusion may be due to a functional disturbance of the alveolar epithelium as proposed by Brauer as an explanation for the cyanotic skin color in certain cases of bronchopneumonia without organic pathological findings This (hypothetical) condition Brauer has termed pneumonosis, analogous to the term nephrosis, which Friedrich

Muller introduced to indicate certain kidney conditions Schjerning has produced lung edema in cats and dogs by intravenous injection of chloramine and subcutaneous injection of urethane and also by chlorine inhalation Blood gas analyses showed incomplete oxygenation of the arterial blood In two dogs the anatomical changes in the lungs after chlorine inhalation were so slight that he assumes a functional disturbance of the epithelium (a pneumonosis) as the cause of the incomplete arterial oxygen saturation In human lungs the condition of a diminished diffusion has so far not been experimentally proven Diagram B in figure 2 indicates in a schematic way the relationship between reduced and oxygenated hemoglobin in the various parts of the circulatory system if the diffusion through the alveolar walls is diminished

The effects on the organism of incomplete oxygenation of the arterial blood

After having described the existence of incomplete oxygen saturation of the arterial blood and considered its causes, it remains to discuss its effect on the organism Unfortunately studies correlating blood gas analyses with clinical symptoms are still so few that it seems for the time being impossible to obtain sufficient insight by these means alone For many years however, physiologists have studied experimentally similar but much less complicated conditions These investigations may prove of benefit to us and serve as a starting point for a discussion of the complicated conditions which we face when trying to unravel the influence on the organism of insufficient oxygenation of the arterial blood

The effect of grave disturbances in the oxygen supply

Complete prevention of oxygen supply causes in all higher animals death in a few minutes because of the small amount of reserve oxygen stored in the body Grave but not immediately deadly disturbances in the oxygen supply cause, as shown in animal experiments, marked irritation of the medulla spinalis and medulla oblongata Mathison showed for instance in 1909 in decapitated cats that insufficient oxygen supply causes irritation of the skeletal muscle and the vasomotor muscles Mathison thinks that oxygen lack acts as a stimulus to the

nerve centers, similar to the action of carbon dioxide and lactic acid He challenges the conclusions of Béthe and of Lindhard that oxygen lack exercises its effect by increasing the irritability of the nerve centers Another remarkable effect of oxygen lack is bradycardia This was first shown by Sherrington Later Th Lewis and Mathison showed that the slow heart action was caused by a temporary heart block

Effects of oxygen lack of moderate severity

These effects have been studied in mountain expeditions, in experiments with low atmospheric pressure in air cabinets, in the respiration of air with low oxygen content, in carbon monoxide poisoning, in certain heart diseases, and in severe anemias The symptoms which can be ascribed to lack of oxygen are in principle the same in all these various physiological and pathological conditions, although they may be modified more or less by circumstances of secondary nature, such as the duration of oxygen lack, which we shall discuss later

The results in mountain expeditions and in air cabinet experiments are for historic and physiological reasons particularly fit as examples of the effects of oxygen lack of moderate severity Furthermore the oxygen lack is in these conditions of the same type as in pneumonia in that it is of respiratory origin We shall therefore in the following mainly confine ourselves to these conditions The condition which may be the result of oxygen lack is now generally termed anoxemia This name is a modification of the term anoxyhemia barometrica introduced in 1864 by Jourdanet. By this term Jourdanet expressed that asphyxia of the body tissues which he considered to be present during stay in rarified air or in air with low percentage of oxygen Anoxemia reveals itself through various symptoms discussed below not all of which are of the same importance or of the same constancy

Cyanosis

The most conspicuous symptom is cyanosis For a purely clinical estimation of the presence of anoxemia or of the grade of the condition if present, the cyanotic color is of no less importance than the pallor in anemia or the yellowness in icterus The essential cause of the cyanosis is the dark color of the non-oxidized hemoglobin present in

the blood When the color of the blood in the capillaries becomes sufficiently dark it is recognized as a diffuse color change through the skin as through milk glass Although it is true that the essential cause of cyanosis is the dark color of the reduced hemoglobin, it must not be overlooked that its appearance and degree, if it is present, are influenced by a series of so called modifying factors Of these factors the state of the capillaries is particularly important For that reason one can draw only roughly quantitative conclusions from the intensity of cyanosis as to the degree of oxygen saturation The minimum

TABLE 5

Arterial oxygen saturation at sea level and at the Andes mountains Oxygen tension in alveolar air and in arterial blood at the Andes (After Barcroft, 1922)

INDIVIDUAL	ARTERIAL OXYGEN SATURATION		ALVEOLAR OXYGEN TENSION—THE ANDES MOUNTAINS	OXYGEN TENSION IN ARTERIAL BLOOD—THE ANDES MOUNTAINS
	Sea level	The Andes mountains 4300 meter		
	per cent	*per cent*		
M	95	83–91	56	58
R	97	87 5	59	52
Bi		84		
Bo..	95	82		
M C Q		86	59	57
P		91	55	48
C		87	54	55
M C I		86	56	47
/		86	51	50
V		82 5		50
B		83 5		40

concentration of reduced hemoglobin in the capillary blood which produces visible cyanosis can therefore be stated only approximately. However, ordinarily it is not far from 6 or 7 volumes per cent [2] This corresponds in patients with normal hemoglobin to an arterial oxygen saturation of 80 to 85 per cent One would therefore expect that normal individuals exposed to rarified air at high altitudes would become

[2] It is convenient to express reduced hemoglobin concentration in terms of oxygen saturation since oxygen values are those experimentally determined One cubic centimeter of oxygen combines with 0 75 gram of hemoglobin So that 5 grams of reduced hemoglobin may be expressed as 5/0 75 = 6 " volumes per cent of oxygen saturation " Volumes per cent " expresses, as usual, cubic centimeters of a gas in 100 cc of blood

cyanotic when the arterial oxygen saturation decreases to about 80 to 85 per cent As a matter of fact this was found to be the case by Barcroft and his collaborators in the expedition to the Andes in 1922 They found that a cyanotic color began at about 4000 meters Determinations made at the same altitude showed an arterial oxygen saturation of from 82 to 91 per cent and in most cases around 85 per cent The figures are given in table 5 In 1915 Hasselbalch and Lindhard in an air cabinet experiment at the Finsen Institute in Copenhagen in a normal individual found no cyanosis at a barometric pressure of 552 mm corresponding to an altitude of a little less than 3000 meters If, however, the pressure was lowered to 484 mm equivalent to an altitude of about 4000 meters cyanosis was found as an inconstant symptom.

Cerebral and cerebellar symptoms

Under anoxemic conditions a variety of psychic disturbances are described As in the case of the cyanotic skin color, these symptoms do not appear before a certain grade of oxygen lack is present As a whole it may be ascertained from the reports of various mountain expeditions and air cabinet experiments that such symptoms start at an altitude of approximately 4000 meters, or at a pressure of not far from 500 mm , which corresponds to an arterial oxygen saturation in normals of approximately 85 per cent Most of the psychic disturbances occurring in anoxemia are of an indefined diffuse nature and can hardly be localized to any certain areas in the brain Loss of self control as in alcohol intoxication, has been repeatedly observed, for instance by Barcroft at an altitude of about 4000 meters in the Alps. It has also been described by Zuntz and collaborators By Mosso, and by the expedition to Pike's Peak in 1913, it was noticed that the men often became unreasonable at about the same altitude Haldane, Kellas and Kennaway reported loss of memory and a delirious condition in an air cabinet at a pressure corresponding to 7500 meters altitude Sleeplessness and headache were observed by Lindhard in the cabinet at a pressure of 484 mm (4000 meters) Irritability, headache, nausea and loss of appetite were reported by Barcroft and his associates to be common at about 4000 meters during the

1922 expedition to the Andes Intellectual impairment is mentioned by Barcroft He states that on the Teneriffa expedition he was unable to solve a simple algebraic problem at an altitude of about 3500 meters The impairment of the senses of vision and hearing noted by Haldane Kellas and Kennaway, is also most likely of cerebral nature Cerebellar disturbances, such as dizziness and vertigo, are frequently reported According to Barcroft, they appear somewhat later (at a higher altitude) than the cerebral symptoms Zuntz and collaborators observed in 1901 on the Monte Rosa expedition that vertigo was present, even with closed eyes Because it is reported not to occur under such circumstances in deaf-mutes, Zuntz considers vertigo to be of peripheral nature, through effects on the bulbar nerve centers

Effect on the bulbar centres especially on pulse and respiration

According to Barcroft's experience, excitation of the bulbar nerves, like the cerebellar disturbances, appear somewhat later than the cerebral symptoms Paul Bert has already shown that the pulse rate increased during stay in rarified air and again decreased to normal if oxygen inhalation was given It is worth noting that whereas the increase in the pulse rate is slight during a decrease of pressure from 760 to about 470 mm , a sudden increase occurs when the pressure is lowered beyond this point Now 470 mm pressure corresponds to an altitude which will cause the arterial oxygen saturation of normal individuals to drop to about 80 to 85 per cent

Paul Bert's results, therefore, indicate that a threshold exists for the action of oxygen lack on the pulse rate, similar to the threshold found for the beginning of cyanosis and for the appearance of psychic disturbances The effect of oxygen lack on the pulse rate was later confirmed by others, for instance, Benedict and Higgins, Schneider and Sisco, and Parkinson These investigators showed not only that the pulse rate was increased by lowered oxygen pressure in the inspired air, but also that the pulse rate dropped if air rich in oxygen (90 per cent) was inspired Parkinson's experiments are of particular interest because care was taken to exclude any possibility of autosuggestion The fact that increased pressure in the inspired air lowers the pulse rate is important because it supports the theory advanced by Böhe and taken up later by Landhard, that oxygen lack acts by altering the

irritability of the nervous centers and not as an irritant, like carbon dioxide The pulse rate seldom rises above 100 However, Haldane reports that his pulse rate increased to 136 when the barometric pressure in the air cabinet was rather suddenly lowered to 330 mm (corresponding to an altitude of about 7000 meters) During prolonged stay in rarefied air, or at high altitudes the pulse rate drops to normal in a few days, according to Haldane, Luscher and others It seems most likely that the effect of oxygen lack on the pulse rate is produced through the bulbar center A direct action on the heart, can, however, not be entirely excluded

Of more fundamental importance for the organism as a whole, is the effect of oxygen lack on the respiration This problem has been the object of a considerable number of investigations, and has particularly interested the schools of Bohr, Haldane, and Zuntz As was found to be the case with the appearance of other symptoms of anoxemia, the effect on the respiration becomes manifest only when a certain degree of oxygen lack is reached According to observations of Haldane and Poulton, a distinct increase in ventilation appears when the percentage of oxygen in the inspired air is decreased to about 12 per cent In animal experiments, Frankel and Geppert, and later Terray showed the threshold value to be about 10 5 per cent. Loewy demonstrated a slight increase in the ventilation even at 16 per cent oxygen in the inspiratory air, and a distinct increase was noted when the oxygen percentage was lowered to 10 per cent

The threshold value for the appearance of changes in the respiration is, therefore, about 12 per cent oxygen in the inspiratory air This corresponds approximately to a pressure of 450 mm or to an altitude of about 4000 meters The increase in the ventilation following a sudden change in the barometric pressure, or in the oxygen percentage of the inspired air, is mostly effected by an increase in the depth of the respirations, although a slight or moderate increase in the respiratory rate may also take place If the oxygen pressure becomes very low, a decrease in the depth of respiration may ensue, so that the breathing becomes shallow and rapid It may be recalled that an increased ventilation is much less useful if it is effected by an increased rate than if it is caused by increased depth If the breathing becomes very shallow, it may even become less effective After a stay of longer

TABLE 6

Rate and depth of respiration and total lung ventilation in normal resting individuals breathing air with normal oxygen tension and air with low oxygen tension

NAME	RATE PER MINUTE	DEPTH	TOTAL VENTILATION	LOCALITY	RATE PER MINUTE	DEPTH	TOTAL VENTILATION	LOCALITY	ALTITUDE OR BAROMETRIC PRESSURE
		cc	*liters*			*cc*	*liters*		
Kimer	17 2	365	6 5	Berlin sea level	15	460	8 3	Monte Rosa in the Alps	1,560 meters
Capart	11	411	5 8		12	705	8 5		
Müller	13	446	5 8		8	1,112	8 9		
Haselbalch	9	784	7 05	Copenhagen sea level	10 7	821	8 8	Brandenburgerhaus Austria	3,290 meters
Lundhard	7	862	6 0		8 3	990	8 2		
Haselbalch	6 7	853	5 7		7 1	990	7 0	Air Cabinet at the Finsen Institute Copenhagen	188 mm
Lundhard	5 1	1,018	5 2		5 1	1,138	6 1		186 mm
Maar	11 9	647	7 7		13 05	70	9 1		553 mm.

duration at high altitudes or in air cabinets with low pressure, the effect on the respiration is different The total ventilation is only slightly increased This is almost entirely caused by increased depth the respiratory rate may even decrease

This is seen in table 6 which gives the total ventilation and the respiratory rate in various individuals under such circumstances The first five observations are from the report of Zuntz and his collaborators on an expedition to Monte Rosa Two are from Hasselbalch's and Lindhard's expedition to Brandenburgerhaus, the last three observations in the air cabinet were made by them at the Finsen Institute This difference in the effect on the respiration between sudden changes in the oxygen pressure and longer exposure to low oxygen pressure is important and will be discussed in greater detail later

Besides change in the total ventilation, oxygen lack causes—as previously mentioned—change in the respiratory rhythm to periodic breathing Haldane and collaborators observed this phenomenon on an expedition to Pike's Peak at an altitude of about 4000 meters Haldane and Meakins found, during inspiration of air with low oxygen, periodic breathing appearing when the percentage of oxygen in the inspired air was lowered to 10 to 11 per cent.

It seems, therefore, that this symptom has approximately the same threshold value as the other symptoms previously mentioned.

Effects of oxygen lack on the metabolism

An important question is the behavior of the metabolism under conditions unfavorable for oxygen intake One would naturally expect that the metabolism would be affected earlier and to a greater extent by oxygen lack than any other function From the time of Paul Bert it has been generally supposed that oxygen lack of moderate degree would cause various disturbances—particularly formation of lactic acid—in the intermediary metabolism Determinations of the total respiratory metabolism under such conditions were undertaken by Loewy in 1895 He showed that the basal metabolism, determined by the total oxygen intake, did not decrease during stay in air rarefied to such an extent that various anoxemic symptoms might be present

In table 7 some of Loewy's results are given together with similar observations of Hasselbalch and Lindhard It is, however, not justifiable to conclude that a quantitative normal total oxygen intake necessarily means that the oxygen supply of all the single organs of the body is normal Only a few observations are at hand as to the oxygen intake of isolated organs during decreasing oxygen pressure Verzar, in 1912, in Barcroft's laboratory, investigated the oxygen intake of various organs during decreased oxygen tension of the arterial blood He found the oxygen consumption of the submaxillary gland independent of decreasing arterial oxygen tension (within certain limits)

TABLE 7

Oxygen consumption of normal individuals per minute at different oxygen tension

NAME OF INDIVIDUAL	OXYGEN CONSUMPTION PER MINUTE	BAROMETRIC PRESSURE OR ALTITUDE	LOCALITY
	cc	*mm.*	
Hasselbalch	270	756	Air cabinet at the Finsen Institute Copenhagen
	267	488	
Lindhard	234	756	
	261	488	
Loewy	185	750	Air Cabinet Berlin
	176	550	
	211	435	
	202	360	
Zuntz	228	Sea level	Berlin
	231 9	2990	Col d' Olen Alps
	259 2	4560	Monte Rosa, Alps

Experiments on the gastrocnemius muscle showed that the oxygen intake decreased with decreasing arterial oxygen tension Hence he draws the conclusion that the oxygen tension in the muscle tissue is normally either zero or near zero, at any rate less than 19 mm Experiments on the heart muscle were somewhat difficult to interpret because of inevitable variations in the work of the heart during the experiments Verzar is, however, inclined to think that the conditions in the heart muscle are similar to those he found in the case of the gastrocnemius muscle. As to the kidneys, decreased arterial oxygen tension caused an increase in the amount of oxygen used by the organ

In his book on the capillaries Krogh points out that experiments made in his laboratory by Gaarder also seem to indicate that the oxygen tension of certain tissues may be zero That a decrease, first in the arterial, and consequently also in the capillary oxygen tension, necessarily must result in a decrease in the oxygen supply of these tissues where the oxygen tension normally is zero, is demonstrated by means of this formula, taken after Verzar.

$$Q = d\,(P - p)$$

where Q is the amount of oxygen which passes per unit of time, from the capillary blood to the tissues, d is a constant, P and p the oxygen tension in the capillary blood and in the tissues respectively If P decreases Q can remain normal only if p diminishes parallel with P. This is, however, impossible if its normal value is zero

It is interesting in this connection that muscular fatigue is one of the most constant symptoms in man during stay at altitudes great enough to produce the previously mentioned anoxemic symptoms It seems as if the feeling of fatigue appears earlier (that is at a lower altitude) than the other anoxemic symptoms Barcroft states that symptoms of fatigue usually appear at an altitude of 3000 meters and are always pronounced at an altitude of 4000 meters That it is not simply due to overexertion is seen by the fact that it is present after rest and also in the morning. Whether it is of muscular or of nervous origin is not known

Fatty degeneration as an effect of oxygen lack

It is well known that lack of oxygen may cause fatty degeneration of various tissues It seems important that the myocardium is one of the organs which in anemia are affected earliest and most markedly. The cause of this is generally believed to be insufficient oxygen supply due to the low concentration of hemoglobin in the blood The results of Verzar's experiments are also interesting in that respect It is, of course, worth emphasizing that the heart muscle never is at absolute rest and therefore, even under the most favorable conditions, needs a considerable oxygen supply Fatty degeneration is found not only in anemia (anemic anoxemia), Lewinstein has produced fatty degeneration in rabbits by keeping them under low barometric pres-

sure, and von Schrotter found marked fatty degeneration in the muscles of guinea pigs kept for three days under a pressure of about 300 mm Hg, corresponding to an altitude of almost 8000 meters That fatty degeneration in anemia may develop in a short time is an old clinical experience

It is not very likely that parenchymatous fatty degeneration occurs under the conditions to which members of mountain expeditions and of air cabinet experiments have been exposed

Effect of oxygen lack on the capillaries

Changes in the state of the capillaries are of importance for the regulation of the blood supply to various regions of the body and also for the appearance and degree of cyanosis, because the filling of the capillaries is as pointed out by Van Slyke and myself, undoubtedly the most important modifying factor in the production of cyanosis Krogh and his pupils have investigated the effect of oxygen lack on the capillaries in isolated organs of animals, and Liebesny and Lüscher have studied the condition of skin capillaries in man under various barometric conditions As a whole it seems as if the state of the capillaries is little affected by oxygen lack of moderate degree

Effect of acute and chronic lack of oxygen

It has already been mentioned that the effect on ventilation of low oxygen pressure in the inspired air is more pronounced if the change in the barometric pressure is sudden than if the change is slowly established Similarly the effect on ventilation is much less marked if the individual has been exposed to low barometric pressure for some time A similar difference is noted as to the behavior of the respiratory quotient during a quite acute and during a more chronic stage of anoxemia Haldane and Poulton showed in 1907 that the respiratory quotient would reach a very high figure—between 2 75 and 3 during the initial part of an acute lack of oxygen At the same time the ventilation was very markedly increased This was, however, only transient, after a short time (less than half an hour) the quotient was within normal values and the ventilation not markedly increased Such an abnormally high respiratory quotient (above 1) is met with under various conditions It may be due to a voluntarily produced

hyperpnoea, or it may be due to a converting of carbohydrates into fat. In both instances the increase in the quotient is, however, much smaller. An increase to about 3 can be due only to such a disturbance in the acid-base equilibrium of the blood that the body for some reason or another must get rid of a considerable part of its carbon dioxide This is further demonstrated through the behavior of the tenson of the cabon dioxide in the alveolar air in people exposed to air with low oxygen tension In such persons the alveolar carbon dioxide tension drops down to two-thirds of its normal value in the first days of an air cabinet experiment The fall in the tension goes almost parallel with the decrease in the barometric pressure The final pressure is 450 mm corresponding to an altitude of between 4000 and 4500 meters How are these effects on the respiration to be explained? The increase in the ventilation and the low carbon dioxide tension have until the last few years been attributed to an increase in the hydrogen ion concentration of the blood, that is to say to an acidosis By this acidosis the stimulus of the respiratory center was raised and an increased ventilation ensued As a result of this increased ventilation the carbon dioxide was washed out first from the lungs, thereafter from the blood Consequently the hydrogen ion concentration of the blood would decrease until a new equilibrium was established at a reaction a trifle more acid than normally When this equilibrium was established the ventilation would diminish towards a normal, and the respiratory quotient would reach its usual value The alveolar carbon dioxide tension naturally would remain low This hypothetical acidosis was attributed by Haldane to production of lactic acid, which—as shown by Fletcher and Hopkins in 1907—is produced if a muscle is contracting under anaerobic conditions However, Ryffel failed to find any lactic acid in blood or urine of individuals who were exposed to rarefied air Later Hasselbalch and Lindhard demonstrated—as shown in figure 6—a moderate decrease in the output of ammonia in the urine during stay in an air cabinet at low barometric pressure This has been confirmed by Haldane, Kellas, and Keenaway

Hasselbalch and Lindhard consider the decreased NH_3 output a direct result of an injury to the liver caused by lack of oxygen It seemed natural that such a decreased production of this very impor-

tant base might result in an acidosis They, therefore, attributed the acidosis under such conditions to diminished production of base and not to increased acid production However during the last few years, Yandell Henderson and Haldane have, independently of each other, performed a long series of experiments the results of which have led them to a new theory about the acid-base equilibrium during oxygen lack Their theory is, to a certain extent, in accordance with the view points originally set forth by Böthe and supported by Lindhard's investigations They assume that anoxemia causes an increased excitability of the respiratory center The result of this is necessarily an increased ventilation This results in a washing out of carbon dioxide Thereby is the normal hydrogen ion concentration decreased until the establishment of a blood reaction a little more alkaline than usual is effected In this way a new equilibrium between the respiratory stimulus and the excitability of the center is brought about The result as to the blood reaction is a slight alkalosis Conclusive determinations of the hydrogen ion concentration of the blood are not at hand And it is, as Haldane remarks—not very likely that they can be produced by our present technique, because the respiratory center is much more sensitive than our best apparatus for direct determination of the reaction of the blood

More recently doubt has been expressed by numerous investigators as to the validity of the alkalosis theory of Henderson and Haldane, Gesell, Koehler, Schneider and others Schneider thinks that several experiences from various mountain expeditions can hardly be explained by assuming an alkalosis to be present in the blood Be that as it may, it is at any rate beyond doubt that we must distinguish in anoxemia between a group of symptoms caused by a disturbance of the normal equilibrium between the respiratory center and its stimuli, and another group of symptoms caused by a more permanent disturbance of the oxygen supply of certain organs The first group of symptoms is distinct only if the change in oxygen pressure in the inspired air is very sudden, and it lasts only a short time They constitute what we may call the first phase in anoxemia An analogous condition is met with in changes of the body temperature which is another important constant of the body We have to distinguish between symptoms due to the movement of the body temperature

from one level to another, and symptoms produced after the new equilibrium has been established And just as only sudden changes in body temperature cause sweat and chill, it is only sudden changes in the oxygen tension of the inspired air which cause distinct hyperpnoea and increased respiratory quotient

In addition to the two phases of anoxemia already mentioned, there is a third phase, which is characterized by an acclimatization to the new conditions on the part of the body How this acclimatization is brought about and how soon it can be established we do not know It is present in inhabitants of high altitudes and in patients with certain heart lesions, particularly in heart lesions of congenital nature Under these conditions a distinct polycythemia is present and possibly an increase in the total amount of blood in the body Of the anoxemic symptoms, at least cyanosis is present, about other anoxemic symptoms nothing definite is known bedause these conditions are very incompletely studied in this respect There are a few observations at hand, which seem to indicate that this third phase may be established in a relatively short time In the report of the Himalaya expedition of the Duke of Abruzzi, it is stated that no anoxemic symptoms were present even at an altitude of much above 4000 meters, the usual threshold value for the appearance of anoxemia

In an expedition to Pike's Peak by Haldane and others, it was observed that the anoxemic symptoms, including even the cyanosis, disappeared in about four days This they explained by assuming an oxygen secretion by the lung epithelium On the other hand, experiments are on record (Hasselbalch and Lindhard, Barcroft) where no acclimatization had taken place after about one week. The problem of how the body acclimates itself to anoxemia is of great importance in human pathology, particularly in respect to certain heart, lung and blood lesions In an acute disease, it is presumably of less or no importance, and we shall therefore not discuss it in further detail

Résumé

We have so far in correlating the anoxemic symptoms with the incomplete oxygen saturation of the arterial blood, expressed the oxygen saturation in terms of volumes per cent or of per cent of saturation However, this is justifiable only so far as the anoxemia is

indirectly due to the arterial oxygen deficit To be quite correct all the anoxemic symptoms are directly dependent not on the condition in the arteries, but exclusively on the conditions in the capillaries And all anoxemic symptoms—except the cyanotic color—are dependent not on the amount of capillary oxygen, but on its tension, which together with the oxygen tension in the tissues determines the tissue supply of oxygen For that reason, general anoxemia may occur in all conditions where general capillary oxygen tension is too low The effect of localized low oxygen capillary tension has so far been studied very little Its importance depends partly on the kind of organs affected At present we shall confine ourselves to general anoxemia Barcroft has subdivided anoxemia into three groups according to the secondary causes of the condition (1) anoxic anoxemia, (2) anemic anoxemia, and (3) stagnant anoxemia, i e , anoxemia found in stasis and slow circulation

We have confined ourselves to a discussion of the first type of anoxic or arterial anoxemia, and so far as the experimental parts go, we have discussed only anoxemic conditions caused by low alveolar pressure (compare diagram *B* in figure 2) In principle, all forms of anoxemia are probably equal, and dependent solely on the capillary conditions Therefore, it might seem preferable to correlate the condition of the oxygen in the capillary blood with the clinically observed anoxemic symptoms It is, however, impossible to obtain true capillary blood (which in itself varies from arterial to venous) with our present technique Møller and I found cutaneous blood to be saturated with oxygen to the same extent as arterial blood

Furthermore the oxygen in the arterial blood is more directly influenced by pathological processes in the lungs than is the oxygen in the capillary blood For these two reasons it is not only necessary but probably also advantageous to express arterial saturation in volumes per cent as the physiological basis for an analysis of anoxemic symptoms in lobar pneumonia, where it is unquestionably decreased arterial oxygen saturation which is responsible for the physiological disturbances in the capillaries, which in turn cause the symptom complex called anoxemia In conclusion, I would again call attention to tables 1 and 5 In table 1 are given the figures for the arterial oxygen saturation in a series of patients suffering from lobar pneu

monia Table 5 gives the arterial oxygen saturation in a series of normal individuals at an altitude of about 4000 meters which indicates, as we have tried to show, approximately the threshold value for the appearance of anoxemic symptoms A heavy line on table 1 indicates approximately the oxygen saturation below which we might expect anoxemia in pneumonia

Pneumonia patients showing an arterial oxygen saturation lower than about 80 per cent are therefore under more unfavorable conditions so far as oxygen supply of the tissues goes than normal people at an altitude of about 4000 meters

In order to make it clear to how great an extent the hemato-respiratory functions in a pneumonic patient deviate from normal conditions, I have calculated by means of a formula introduced by Van Slyke and myself the blood fraction which could pass from right to left through a defective ventricular septum of the heart before the oxygen saturation of the mixed arterial blood went below 80 per cent This would, if the concentration of hemoglobin remained normal, be about 33 per cent of the total amount of blood traversing the heart It is quite astonishing that there is present in one-third of the patients in table 1 an arterial oxygen unsaturation greater than that observed in normal individuals who are at an altitude of about 4000 meters or who are exposed to only 14 per cent oxygen in the inspiratory air, and greater than the arterial oxygen unsaturation caused by septal defect large enough to side track one-third of the blood from the lung circulation

Every individual suffering from pneumonia has to struggle with an infection which in itself may be serious enough A number of these patients have to fight under a condition of anoxemia which at best can never be favorable, and which in some instances may be the leak that sinks the ship.

II THE EFFECT OF ANOXEMIA IN LOBAR PNEUMONIA

The problem which we shall now discuss is To what extent can symptoms, occurring in patients with pneumonia, particularly of the lobar type be attributed to anoxemia?

Cyanosis

It was previously stated that the occurrence of the cyanotic color is dependent on the presence of reduced hemoglobin in the capillary blood. Other things being equal, the intensity of cyanotic skin color increases with the increase in concentration of reduced hemoglobin after the threshold value, about 6 to 7 volumes per cent of oxygen unsaturation, has been reached. The other anoxemic symptoms, dependent probably on the supply of oxygen to the tissues, presumably depend on the fall in the arterial oxygen tension in the capillary blood. After the threshold has been passed, these symptoms increase in intensity with decreasing oxygen tension. In normal individuals with normal concentration of hemoglobin in the blood it so happens that the threshold for the appearance of cyanosis and for the appearance of other anoxemic symptoms is passed at approximately the same time. The result of this is that the presence or absence of cyanosis indicates whether the physiological condition (i.e., a sufficiently low capillary oxygen tension) for the development of the other symptoms is present or absent. It is justifiable to assume that in most patients with pneumonia a similar parallelism between cyanosis and other anoxemic symptoms is present. The reason for this is that both abnormalities (cyanosis and other anoxemic symptoms) are the result of the same pathological conditions, namely, the decreased arterial oxygen saturation, which is in turn caused by pulmonary insufficiency. To the same extent that this parallelism is present it is fair to consider cyanosis a symptom of general anoxemia. Although this is in main true, certain exceptions must be kept in mind by the clinician who wishes to use the cyanotic color as a diagnostic guide.

Cyanosis may be present without being associated with general anoxemia in several conditions, which fall into four groups. (1) If the cyanotic color is a purely local phenomenon due to abnormally increased deoxidation of the normally saturated arterial blood. Such a local microcyanosis is not very uncommon even in otherwise normal individuals and it can be assumed that it also may be found now and then in patients with pneumonia. (2) If the cyanosis appears at a smaller concentration of reduced hemoglobin in the capillaries than usual, due to an increased influence of the so called modifying factors, stasis, increased number of blood filled capillaries, etc. It has been

generally assumed that a cyanotic color in pneumonia was due in the majority of cases not to respiratory but to circulatory disturbances, that is to an insufficiency of the heart If this were true, stasis and increased peripheral deoxidation should be looked upon as the most important factors in the production of cyanosis in pneumonia. However, clinical investigations by Petren and determination of the blood gases in pneumonic patients by Stadie, le Blanc and myself have shown that a more than purely local cyanosis found in pneumonia is practically always due to respiratory disturbances, which through insufficient oxygenation give rise to abnormally decreased arterial oxygen saturation (3) Cyanosis may be observed without the presence of other anoxemic symptoms in patients with *chronic* cyanosis. This is seen in certain congenital heart diseases and probably also in some cases of emphysema (4) Cyanosis without anoxemia might be caused by the so called Bohr phenomenon Bohr and his associates Hasselbalch and Krogh made in 1904 an observation which became of fundamental importance and is often referred to as "Bohr's phenomenon" They showed that increased carbon dioxide tension of the blood would cause an increased tension for the same amount of oxygen present

If, in pneumonia, an increased hydrogen ion concentration, an acidosis, occurred at the same time as an insufficient arterial saturation it might be assumed that oxygen tension in the capillaries might be kept sufficiently high to secure an adequate oxygen supply to the tissues even if the concentration of reduced hemoglobin in the capillary blood had passed the usual threshold for the production of cyanosis This problem, important in heart disease especially in relation to treatment with carbon dioxide inhalation seems purely theoretical so far as pneumonia goes, because Hastings, Morgan, Binger and Neill have shown that if any change takes place in the reaction of the blood in pneumonia, a very slight alkalosis is found

In the conditions encountered in these four groups, anoxemic symptoms (except cyanosis) may be lacking either because the decrease in the tension is too small, or because it affects too few organs of importance, or because the organism in some unknown way, as in chronic cyanosis, acclimatizes itself to the abnormal conditions In acute pneumonia patients without heart insufficiency, emphysema or poly-

cythemia, a general cyanosis indicates that the arterial oxygen saturation has decreased to 85 to 80 per cent and that the pathological conditions essential for the production of the other anoxemic symptoms may be expected to be present

On the other hand cyanosis may be absent in cases where other anoxemic symptoms are very marked, these cases fall into two groups First, this is seen in severe anemias It has been shown that cyanosis does usually not develop in patients with extreme anemia because the concentration of reduced hemoglobin cannot pass the average threshold value for the development of a cyanotic skin color And the more marked the anemia (and therefore the anoxemia) the less marked is the tendency to cyanosis, a fact which of course is of great importance for the functional diagnostic value of cyanosis in anemic conditions However, a presence of anemia is easily recognized either by simple observations or by a determination of the hemoglobin Secondly anoxemia without cyanosis is theoretically possible in cases of alkalosis This is due to the Bohr effect Due to a very low carbon dioxide tension in the blood the oxygen tension may become abnormally low even if oxygen content is normal For that reason the capillary blood may be unable to give off sufficient oxygen to the tissues A condition of alkalosis is rare It has only been observed once (Binger, Hastings and Neill) in connection with pneumonia, and in this case it was due to administration of bicarbonate of soda to the patient over a considerable time In carbon monoxide poisoning a somewhat analogous condition is met with and the skin color may be even more red than usual in spite of dangerous anoxemia It is therefore justifiable to state that absence of cyanosis in patients with pneumonia excludes anoxemia unless the case is complicated with severe anemia, alkalosis, or carbon monoxide poisoning *The absence or presence of cyanosis, therefore, indicates in the majority of pneumonic patients the absence or presence of the physiological conditions necessary for the development of most other anoxemic symptoms For that reason and because cyanosis is easily recognizable without any special apparatus, it is a symptom of great fundamental diagnostic importance in pneumonia*

Frequency of cyanosis in pneumonia

That it is of great importance to ascertain how frequently a cyanotic color is present in pneumonia is self evident So far relatively few

observations on this question have been published In 1239 cases collected from the literature, Norris found cyanosis in 119 cases (10 per cent) Lyon found the symptom in 9 per cent of 109 instances of lobar pneumonia in childhood The mortality of these cases was 4 5 per cent In 658 cases, collected from Johns Hopkins Hospital, Chatard found, as shown in table 8, cyanosis in 33 per cent A marked cyanosis was present in 12 per cent The total mortality in Chatard's cases was 31 per cent Of the cyanosed patients 43 per cent died

TABLE 8

Frequency of cyanosis and changes in respiration in 658 patients with lobar pneumonia in the Johns Hopkins Hospital, Baltimore, Md (Chatard)

	NUMBER		NUMBER	PER CENT	NUMBER DIED
Cyanosis	658	Mild	134	20 3	58
		Pronounced	81	12 2	35
Character of respiration	658	Dyspneic	367	55 6	140
		Strenuous	165	25 1	71
		Shallow	35	5 3	20
		Periodic	6	0 9	5
Respiratory rate	643	30	48	7 5	4
		30–40	208	32 3	31
		40–50	192	29 9	56
		>50	195	30 3	107

According to Cole cyanosis is an almost constant symptom in all severe cases of lobar pneumonia De la Camp and Morawitz in their articles on pneumonia in Kraus and Brugsch's and in Mohr and Staehelin's handbooks respectively do not make any statements as to the frequency of cyanosis in pneumonia It would be highly desirable to obtain accurate numerical information about the frequency of cyanosis in pneumonia and its relation to the other symptoms and to the various anatomical states of disease, If one should try to estimate the frequency of cyanosis in pneumonia from the rather incomplete information now at hand, one would say that it is present in at least 10 per cent of all cases This figure is, however, undoubtedly too small The mortality of the disease is approximately 20 to 25 per

cent and in almost all these cases cyanosis occurs although probably not always due directly to respiratory insufficiency Cyanosis, usually of respiratory origin is furthermore met with in a number of patients who recover It is probably justifiable to assume that cyanosis (of respiratory origin) occurs in about 20 per cent of all patients with lobar pneumonia

The clinical recognition of cyanosis

We shall not in this place enter into details concerning the clinical recognition of the presence and degree of a cyanotic color This is best discussed in connection with bedside observations Emphasis is required on the fact that in deciding the question of the presence or absence of the physiological conditions for development of anoxemia one can find help from the presence or absence of cyanosis only if the clinical observation of the skin color is done as carefully as the stethoscopical examinations of the lungs

Psychic disturbances in pneumonia

As to the occurrence in pneumonia of cerebral and cerebellar anoxemic symptoms, only very little and not very precise information is yet available in the literature Psychic disturbances of various kinds are by no means infrequently seen in patients with pneumonia In the more severe cases such symptoms are almost always observed often in a very pronounced degree These symptoms are as a whole of the same rather indefinite nature as those found in experimental anoxemia It is, therefore, at any rate not impossible that anoxemia may play a causative rôle in the development of these symptoms On the other hand similar symptoms may be observed in other acute infectious diseases, where there is no disturbance in the oxygen supply

The psychic disturbances encountered in pneumonia are usually of a delirious character, they may even be so pronounced that the patients leave the bed and try to jump out of the windows And even if patients severely ill from pneumonia, are not absolutely delirious, they are very often unreasonable and show a marked lack of judgment Insomnia, excitability, and a certain kind of apprehension are not infrequent

In the so called asthenic pneumonia, a name used first by Leichtenstein in 1873 to designate a certain atypical form of pneumonia, psychic disturbances are almost constantly present. A subdivision of this group is even termed the cerebral form of pneumonia These atypical forms differ in several respects from the usual type of lobar pneumonia Their etiology is often and their pathology almost always different. A number of cases of asthenic pneumonia therefore are not of the lobar type, many do not show a fibrinous exudate in the alveoli and most of them are probably not caused by pneumococcus Cases of influenzal pneumonia usually belong to this asthenic form. It is of considerable interest in regard to our problem that marked cyanosis and pronounced psychic disturbances are found together in a very large number of these patients. And it is a rule that the more marked the cyanotic color the more pronounced is the psychic disturbance It is of importance that a few observations are on record where psychic symptoms disappeared or became less marked during oxygen inhalation therapy. Meakins, Barach and Woodwell, and Stadie have described such cases where the effect of oxygen therapy suggests strongly that psychic disturbance in pneumonia patients, such as delirium, excitability and insomnia may be of anoxemic nature. It is to be hoped that if interest becomes centered on this important question, future clinical observations may bring clarity into the problem of to what extent psychic disturbances in pneumonia patients are due to anoxemia.

Pulse rate and anoxemia

In view of the fact that the increase in pulse rate observed in experimental anoxemia is rather small and usually transient, it is *a priori* not very likely that increase in pulse rate in pneumonia patients is due to anoxemia We know, furthermore, that an increased pulse rate by no means is confined to cases showing cyanosis, which is in most cases, as we have pointed out, our clinical criterion of the presence of the physiological conditions necessary for a development of anoxemia However, the fact that a drop in the pulse rate has been observed during oxygen treatment suggests that anoxemia may in some instances be partly responsible for the increase in pulse rate. Stadie observed for instance a drop in pulse rate from 160 to 120

following effective oxygen treatment Whether this effect is due to an action on the bulbar center or whether it is a direct effect on the myocardium is unknown

Peripheral circulation

Besides being of importance for the efficiency of the circulation, the behavior of the (skin) capillaries is of moment for the development of cyanosis The few observations hitherto made on the capillaries in experimental anoxemia make it unlikely that any abnormalities which future investigations may reveal in the behavior of capillaries in patients with pneumonia can be ascribed to anoxemia.

Respiratory disturbances

The behavior of the respiration in pneumonia is of the greatest scientific and practical importance We face at the same time a demand for an increased respiratory activity and a more or less impaired functional power of the lungs An increase in the respiratory rate is well known to be one of the most prominent symptoms in pneumonia If compared with pulse rate or temperature elevation the increase in respiratory rate is less marked in most other febrile diseases In table 8 is given the frequency of respiration in 658 pneumonia patients collected by Chatard Is this pronounced increase in respiratory rate entirely or partly an anoxemic symptom? That it is not solely due to an increased irritability of the respiratory center through oxygen lack may be concluded from the fact that an increased rate of respiration is observed also in non cyanotic patients And in most of these cases we may assume that even if an oxygen lack is present it has not reached the intensity required for development of anoxemic respiratory symptoms Furthermore there is a marked and very important difference in the character of the respiratory disturbances observed in experimental anoxemia and in pneumonia

In anoxemia produced in normal individuals by inspiration of air with low oxygen the change in respiration is, as shown in table 6, usually not very pronounced and mainly characterized by an increased depth of respiration In pneumonia it is different The depth of respiration is either normal or—most frequently—diminished An increased ventilation is in almost all instances due to an increased

rate The importance of this difference for the efficiency of the ventilation is extreme, as previously pointed out Does that difference in the mechanism of the increased ventilation in experimental anoxemia and in pneumonia prove that the respiratory stimulus is different in the two conditions? Not necessarily, because it is natural to expect that local pathological processes present in the lungs of pneumonia patients might in some way or another modify the response of the respiratory center to the same abnormal condition in the blood (decreased oxygen tension). Several possibilities suggests themselves In the first place one might consider the mechanical conditions within the lungs Is it not possible that diminished lung volume and impaired mobility simply prevent pneumonia patients from increasing the respiratory depth or even from retaining the normal depth? It seems *a priori* natural However, observations are at hand which point strongly against this explanation We know that processes of non-pneumonic nature may encroach upon the air spaces and hamper the mobility of the lungs without preventing an increased depth of respiration under conditions where such an increase is demanded It has furthermore been observed by Binger and Brow that the depth of respiration may increase considerably immediately after crisis, that is at a time when the consolidation is still present in the lungs In the second place one might attribute the superficial respiration to pain which we know as a cause of superficial respiration in cases of, for instance, pleurisy, injury to the chest-wall, etc The fact that morphine in some cases of pneumonia may increase the depth of respiration might also point to pain as the cause. However, various circumstances point against this explanation Superficial inspiration is by no means confined to patients in whom respiration is painful (usually on account of pleurisy) Shallow breathing does not cease during sleep, which shows that the shallow breathing is at any rate involuntary. Recent studies of the action of morphine on the respiration from Richard's laboratory seem to offer another explanation of the influence of morphine on respiration sometimes seen in pneumonia patients

Various investigations, mostly of recent years, suggest strongly another way in which local pathological processes in the lungs may influence the respiration and thereby profoundly alter the response of the respiratory mechanism to either a normal or abnormal hormone

stimulus It might be helpful in the discussion of this problem to recall a few important points in the historic development of our conception of the maintenance of the respiratory rhythm Three main periods are more or less distinct Galén considered the respiratory rhythm to be an original voluntary act which has become a habit The second period developed with nerve physiology In this period in the first place the existence of a respiratory center in the medulla oblongata was recognized, in the second place it was shown that the activity or state of this center was influenced by various nerve impulses of reflex nature By means of these reflexes the respiratory rhythm was maintained in its normal state or eventually altered The most important of these reflexes was the so called Hering-Breuer reflex According to investigations by Hering and Breuer in 1868 and by Head in 1889 the respiratory rhythm is maintained simply through the action of two centripetal nerve fibers in the lung vagus These nerve fibers act on the respiratory center like a switchboard and initiate inspiratory and expiratory movements respectively according to the dilatation or compression of the alveoli

The third period reached its summit when the conception of hormonal action became introduced into medicine It is characterized by a tendency to consider respiration to be almost exclusively regulated by stimuli carried to the respiratory center by the blood This conception can be traced as far back as 1862 to two observations, which later on were recognized to be of fundamental nature One was Rosenthal's demonstration of the cessation of respiration after the forced deep respiration, a condition which he termed apnea The other was Traube's observation that apnea might be produced also if hydrogen or nitrogen was inspired This led Traube to consider carbon dioxide the stimulus to the respiration However, it was first after the quantitative demonstration by Haldane and Priestley in 1905 of the effect of carbon dioxide on the respiration that these viewpoints gained ground and began to inspire people to further investigation of the problem

A further support was given this theory by the demonstration, by Hasselbalch and myself in 1912 of the possibility of explaining this effect of carbon dioxide through changes in the hydrogen ion concentration of the blood which according to our view should be considered

the adequate stimulus of the respiratory center At the same time great progress was made in biological chemistry and it was quite natural that a tendency should prevail to ascribe most respiratory disturbances exclusively to changes in the reaction of the blood The interest in the influence of the nervous system on the respiration was rather small in that period However, the importance of hormonal stimulus for the activity of the respiration undoubtedly has been overrated at the expense of the stimulus of nervous origin. In the first place various pathological phenomena cannot be explained solely as a result of hormone changes, and second, several important observations point directly to a greater influence than usually assumed, on the respiratory changes from stimuli through the nerves and in particular through the vagi Alcock and Seeman demonstrated in 1905 the presence of a demarcation current in nervi vagi even during normal respiration Scott showed in 1908 that hormone stimulation of the respiration had a different effect according to whether the lung vagi were intact or severed In the first case a hormone stimulation of the respiratory center increased the ventilation through increased depth and through increased rate as well In the last case only an increased depth was seen These experiments suggest that hormonal stimulus from the blood increases the depth whereas stimuli travelling through vagi from the lungs affect the frequency The nerve stimuli probably cut off the depth before the distension of the lungs has reached the limit set by the mechanical structure of the chest

Of particular interest for our conception of the influence of the vagi if pathological processes are present in the lungs are a series of investigations by Porter and Newburgh. They found that increased depth of respiration, which normally is the result of carbon dioxide inhalation, could be demonstrated in pneumonic dogs if the vagi were cut They also showed that dogs suffering from pneumonia did not show any marked increase in the respiratory rate if the vagi were cut In a later publication they demonstrated that the respiratory rate in pneumonic dogs would drop from 60 to about normal if the vagi were cocained In Porter and Newburgh's experiments pneumonia of lobar type was produced by the Friedlander bacillus The problem, why increased lung ventilation in some conditions—as for instance,

diabetic acidosis and moderate anoxemia—is effected mainly through increased depth and in other instances—as for instance in pneumonia, and in some heart cases during excessive exercise—is effected mainly through increased frequency, is undoubtedly a very complex one

It seems almost certain that processes in the lungs of inflammatory origin may through the nervi vagi affect the respiratory center in such a way that the answer to an increased hormonal stimulus solely becomes increased frequency of respiration instead of merely increased depth One must furthermore assume that a pathological process in the lungs may change the answer to the normal stimulus, so that the frequency of respiration may increase before the oxygen lack has passed the threshold value for increasing the excitability of the center In this way shallow breathing might be the cause of anoxemia in pneumonia on account of the previously mentioned ineffectiveness of a respiration of small depth A combination of impaired functional effectiveness of the ventilation probably caused mainly by a reflex from the pathological processes in the lungs and of increased hormonal stimulation of the respiratory center indirectly caused by the lowered oxygen tension in the blood may prove a very serious, at times even a disastrous condition to the patient Through this a vicious circle may—as clearly pointed out by the English school—be established an oxygen lack, just above the threshold, which in a normal individual increases only the depth of ventilation slightly, may, in a patient with pneumonia, increase the frequency and decrease the depth of respiration to such an extent that insufficient alveolar ventilation resulting in further decreased arterial oxygen saturation may ensue This vicious circle the patient himself cannot, and the physician only with difficulty, break

It has been mentioned that periodic respiration is almost constantly seen in experimental anoxemia in normal individuals According to Chatard's clinical observations (table 8) this symptom is very rare in pneumonia No explanation can be offered for this fact It seems most likely that it is due to a reflex action of the respiratory center from the lungs This symptom, clinically just as easily recognized as cyanosis therefore becomes of very little functional diagnostic value in regard to the presence of anoxemia in pneumonia

Oxygen metabolism in pneumonia

It may be recalled that the basal metabolism expressed in terms of oxygen intake was not diminished in experimental anoxemia On the contrary an increase of about 20 per cent in the total intake was usually observed. It is natural to assume that an anoxemia of the same intensity does not interfere with total oxygen intake in pneumonia, not even in view of the fact that the fever increases the demand to a certain extent However—as previously pointed out—a normal or even increased total oxygen intake, does not prove that every single organ in the body is properly supplied with oxygen It was also pointed out that it is very likely that for instance the oxygen supply of the muscular system decreases when the capillary oxygen tension drops beyond a certain value. If in pneumonic patients similar impairment of oxygen supply to certain organs occurs it is of particular importance to two muscle groups the respiratory muscles and the myocardium, because the demand on both is greatly increased We shall in this lecture consider only the importance of oxygen lack to the function of the myocardium. In order to treat this obscure question intelligently it is necessary to enter into a general discussion of the function of the heart in pneumonia.

The function of the myocardium in pneumonia and its relation to anoxemia

It is generally assumed that the most direct cause of death in pneumonia is a breakdown of the circulation through heart failure. An inquiry into the literature as to the condition leading to heart failure in pneumonia yields very little and rather indefinite information Some points may be discussed We know that the strain on the heart muscle is increased above normal for two reasons first, on account of the increased temperature and metabolism, and, second, on account of increased resistance in the pulmonary circulation Whereas the first of these reasons is evident, the last needs further discussion Lichtheim drew from his well known experiments in 1876 the conclusions that the area of the pulmonary circulation could be reduced to about one-fourth before any increased circulatory resistance occurred Lichtheim inferred from his demonstration that the arterial blood pressure was maintained in his experiments Rosen-

bach criticized Lichtheim's conclusions and pointed out that the arterial blood pressure in his experiments might have been kept up through compensatory vasomotor constriction and not through passage of a normal amount of blood through the lung remnants One may add that if the pulmonary circulation had remained quantitatively normal in Lichtheim's experiments, his results could be explained by assuming that the heart by using its reserve force had overcome an increased resistance in the pulmonary circulation

At any rate in lobar pneumonia there can be little doubt as to the occurrence of an increased resistance in the pulmonary circulation In the first place we know that the vessels in the afflicted parts of the lungs are considerably dilated On account of lack of tonus the dilatation can be caused by nothing else than an increased pressure, secondly, the second pulmonic sound is usually accentuated in patients unless a heart failure is present Thirdly, there is an increased frontal area of the heart on x ray examination

This last phenomenon shown by Dietlen and by Levy may be a physiologic condition caused by an increased pulmonic pressure analogous to what has been found experimentally by Starling, who demonstrated a dilatation of the left heart when the arterial pressure was increased Or it may be a pathologic condition indicating a beginning myocardial failure

To what extent the two factors mentioned (i e, the increased metabolism and increased pulmonary resistance) may exhaust the reserve force of the myocardium is unknown However, experience from other pathologic conditions, where a similar increase in the metabolism or in the pulmonary blood pressure is encountered, make it unlikely that these two factors may be able to exhaust in a few days the reserve force of a normal and normally nourished myocardium

It seems therefore necessary to assume either an anatomical or a functional injury of the myocardium in cases of pneumonia where a circulatory failure is encountered

As to the first point not very extensive information is at hand The few investigations hitherto published concerning the anatomical condition of the myocardium in pneumonia show that an injury of inflammatory or degenerative nature is not frequent It is also of

importance that Romberg and collaborators were unable to detect any anatomical injury in the myocardium of animals infected with pneumococci

Heart failure in pneumonia must therefore mainly be ascribed to a functional disturbance of the myocardium

A functional disturbance of the myocardium may be produced by two factors· toxemia or anoxemia

Toxemia In an infectious disease one naturally expects toxemia to play an important rôle in the breaking down of the circulation The fact that no circulating toxin has so far been demonstrated in pneumonia can not exclude a toxic injury of the myocardium

Newburgh and Porter have shown that the contractile power of normal myocardium diminished when transfused by blood from dogs in which pneumonia was produced by Friedlanders bacillus When the same experiment was performed on myocardium from pneumonic dogs no difference was found between the contractile power during transfusion with normal and pneumonic blood They drew the conclusion that the myocardial tissue in some way or another had accommodated itself to the hypothetical pneumonic toxin

Anoxemia As to the importance of anoxemia in producing a functional impairment of the myocardium very little experimental or clinical evidence can be found. Experiences gained at high altitudes and in air cabinets show that anoxemia alone does not seem to impair the circulation during rest On the other hand it is well known that even vigorous individuals perform only with difficulty exercise at low atmospheric pressure

Experiments on fish embryos (Loeb) and frogs (Weizacker) show that diminished oxygen supply may impair or even stop the contraction of the myocardium None of these experiments are quantitative in the sense that the oxygen tension during the experiment was determined They can therefore only give suggestions Loeb found in certain fish embryos that the contraction rate of the heart decreased during insufficient oxygen supply In some instances the rate decreased regularly to a certain point at which it stopped In other instances the heart continued for hours at a slow rate In a third group the rate fell regularly to almost zero Weizacker transfused

frogs' hearts with blood, the oxygen content of which was steadily diminished to about one-third of its normal value He found little or no decrease in the work performed by the heart However, no remarks are found in Weizacker's experiments about the oxygen tension of the blood, which is the main factor governing the oxygen supply And there is a possibility that carbon dioxide produced during the experiment may have increased the tension of the oxygen (Bohr's phenomenon)

Clinical experience concerning the effect on the heart functions of disturbances in the oxygen supply are very scanty and far from quantitative Extra systoles, auricular fibrillation and heart block, not infrequently observed in pneumonia may possibly in some instances be caused by anoxemia In other and probably more instances the cause may be toxemia Occurrence of heart block in pneumonia is of particular interest because it is experimentally shown that heart block may be caused by oxygen lack

In 1915 I observed a transient attack of heart block in a cyanotic patient with pneumonia Attacks of heart block are most frequently seen in the convalescent stage, i e, at a period where no anoxemia is present Attention has been called to the dilatation of the heart found by Dietlen and by Levy, and it was mentioned that such a dilatation may indicate a functional disturbance of the myocardium

In spite of the lack of experimental and clinical information about the functional condition of the myocardium in pneumonia it seems justifiable to expect that insufficient oxygen supply of the myocardium may in some instances cause a breakdown of the heart, especially if it is injured by toxins and hampered by increased resistance

The adaptation of the pneumonic organism to anoxemia

We have divided the effects of the experimentally produced anoxemia into three groups first, a series of symptoms indicating the endeavour of the organism to adapt itself to the new condition This phase was analogous to what happens when an organism is trying to reach an equilibrium at a temperature higher than the normal The second group of symptoms represented the symptoms which prevailed after the equilibrium had been reached The third phase represented the slow accommodation of the organism to a

chronic anoxemia seen in certain heart diseases or during long lasting exposure to low barometric pressure

We have so far in our discussion of anoxemia in pneumonia mainly discussed the second phase However, the question about the first phase is an important problem in pneumonia for the reason that it is

TABLE 9

Oxygen saturation, carbon dioxide tension of arterial blood, and body temperature in 8 patients with lobar pneumonia at Rockefeller Hospital, (Binger, Hastings, Morgan and Neill, personal communication)

NUMBER	OXYGEN SATURATION OF ARTERIAL BLOOD	TENSION OF CARBON DIOXIDE IN ARTERIAL BLOOD	BODY TEMPERATURE
	per cent	*mm* Hg	
3		36 9	38 7
		45 6	37 7
4	84	37 5	39 6
		36 5	38 8
		40 3	37 3
5	68 2	44 7	37 8
	97 2	34 8	39 6
8	64 2	42 3	37 4
	90 5	35 1	39 6
9	94 4	40 3	39 4
	97 0	44 6	37 3
13		34 8	40 2
	89 7	40 7	37 3
14	91 8	40 3	37 4
	96 2	33 3	39 7
15	68	42 8	39 8
	81	40 3	40 2
	84	37 3	37 3

mainly by means of the respiratory function that an organism reaches a new equilibrium. One should naturally expect that the conditions in a pneumonic patient are unfavorable in that respect The question is· Do pneumonic patients suffering from anoxemia wash out part of

the carbon dioxide of the body in order to lower the hydrogen ion concentration and thereby protect the respiratory center? A few determinations of the carbon dioxide content of the blood in pneumonia are at hand As a whole the values are below normal But as the important factor is the carbon-dioxide tension and not the content these determinations are of only suggestive value because the content and the tension of this gas do not necessarily run parallel Reliable determinations of the carbon dioxide tension of the alveolar air in pneumonia are naturally not at hand

In table 9 are given the results of a series of determinations of the carbon dioxide tension of the blood in pneumonia done in 1923 in the Rockefeller Hospital

In most cases the arterial oxygen unsaturation was also determined As a whole no considerable decrease in the carbon dioxide tension is found At any rate the decrease is much less than that found in normal people with experimental anoxemia of the same degree Furthermore no parallelism can be demonstrated between the—moderate—decrease in the carbon dioxide tension and the degree of arterial oxygen unsaturation The decrease in the carbon dioxide tension of the blood is therefore not to be looked upon as a washing out of the carbon dioxide on account of anoxemia

There seems to be no doubt that the low carbon dioxide tension is related directly or indirectly to variations in the body temperature, an important fact demonstrated originally by Frederica and Olsen in the alveolar air of patients with febrile diseases

Determination of the hydrogen ion concentration of the blood in the patients in table 9 showed values[1] either normal or slightly alkaline These determinations show either that pneumonic patients with anoxemia are unable to lower the carbon dioxide tension of the blood or that such patients for some unknown reason do not need this regulation which, as previously mentioned, is present in normal individuals exposed to anoxemia In the first case one should expect a permanent overexcitation of the respiratory centre the excitability of which is increased through the anoxemia In the second case one must assume that the kidneys or the liver have taken over that part of the regulation which the diseased lungs cannot perform.

[1] Not given in table 9

The third phase, the effect of chronic anoxemia on the body, will not be discussed here because it is undoubtedly of little or no importance in an acute disease as lobar pneumonia.

Oxygen treatment of pneumonic patients

The results of oxygen treatment of pneumonic patients will be mentioned only so far as they are of importance for the elucidation of anoxemic symptoms During the last four or five years about 40 cases have been published in which the treatment, the blood gas determinations, and the clinical description are such that the results may throw light on the question of the anoxemic symptoms in pneumonic patients

In about 60 per cent of the cases the oxygen content of the arterial blood has been increased to normal values This very important fact shows that only in 40 per cent of the patients is the abnormally low arterial oxygen saturation caused by perfusion of consolidated lung areas inaccessible to air

In most instances cyanotic color has disappeared when the abnormal oxygen unsaturation was abolished, showing as previously mentioned that cyanosis in pneumonia usually is of respiratory origin In a case published by Woodwell and Barach a moderate acrocyanosis was present even after the oxygen content of the arterial blood had become normal In this case the cyanosis must be attributed to increased peripheral deoxidation of the capillary blood through stasis Also other symptoms of circulatory failure were present

In several patients where pronounced cerebral symptoms were observed, Meakins, Barach and Woodwell, and Stadie found that after oxygen therapy these symptoms either disappeared entirely or became less The patients became less delirious and quieter and the sleeplessness disappeared In some instances the effect of oxygen treatment on the psychic disturbances has been remarkably striking The pulse rate is usually not much influenced by oxygen therapy In a few patients slowing of the pulse rate does occur and in one of Stadie's cases a drop from 160 to 120 was observed when the anoxemia was abolished Whether or not this effect may be ascribed to a better oxygen supply of the myocardium we do not know.

The effect of oxygen therapy on the respiratory rate is of importance for our conception of the nature and pathogenesis of the dyspnoea in pneumonic patients In most cases successful oxygen therapy has been followed by only a small drop in the respiratory rate, an observation which is in accordance with our conclusions in the chapter on the respiration in pneumonia It is clear that if an increased sensitivity of the respiratory centre, caused by decreased blood oxygen tension, were the cause of the dyspnoea, this condition should be alleviated or abolished when the blood oxygen became normal The results of the oxygen therapy gained so far, therefore, support the conception that the main cause of the dyspnoea in pneumonic patients is a reflex from the local processes in the lungs

The results of oxygen therapy do not help us to decide whether or not a restoration of normal arterial oxygen unsaturation has any effect on the function of the myocardium The fact that we do not possess any suitable functional test for the heart makes it improbable that we shall gain any information before a very large amount of material is collected or before experiments on isolated hearts are performed

The material is also too small and clinically too heterogeneous to yield any information about the influence of oxygen therapy on the course of the disease

Résumé

The term anoxemia may at present appear strange and unreal In a few years however it will be as familiar as uremia This last term is in several respects similar to anoxemia but much more obscure in spite of the fact that for a long time it has been a part of the pathologic system Both terms comprise groups of symptoms and not etiologic entities, and both are a result of severe impairment of the function of important organs But whereas uremia in the majority of instances indicates a desperate terminal condition caused by irreversible anatomical disturbances, anoxemia in pneumonic patients is caused by a disease where the possibility of complete recovery is present It is therefore of the greatest importance to treat this symptom as soon and energetically and above all, as scientifically as possible

Summary

The main points developed above are the following

1. In 10 to 20 per cent of patients with lobar pneumonia disturbances in the respiratory functions of the lungs cause an abnormally low oxygen saturation of the arterial blood This in turn gives rise to a decrease in the amount and tension of the oxygen of the capillary blood

2 Through physiological and pathological investigations it is demonstrated that when a decrease in the arterial oxygen content (and consequently also in the oxygen content and tension of the capillary blood) exceeds a threshold of 15 to 20 per cent a group of pathologic symptoms appears in normal individuals The main symptoms are· Cyanosis, mental disturbances, abnormal irritation of the nervous centres in the medulla, particularly of the respiratory centre, probably an impairment of the function of the muscular system, particularly of the myocardium

The term anoxemia is in most instances used to indicate the condition characterized by these symptoms In other instances anoxemia simply means the underlying pathologic condition, namely the incomplete oxygen content of the blood

3. An attempt has been made to show by analyses of different facts published in the literature that the various anoxemic symptoms in the main appear at the same threshold level of arterial oxygen unsaturation The presence or absence of a cyanosis may therefore in most instances serve as a clinically useful criterion for the presence or absence of an arterial oxygen deficit extensive enough to give rise to other less characteristic anoxemic symptoms

4 In pneumonia similar symptoms are frequent A deeper analysis of these symptoms shows first that anoxemia probably plays a part in their development, secondly that anoxemia is to be looked upon only as one of the causes of these symptoms To what extent some of the most important functional disturbances in pneumonia, namely the dyspnoea and heart failure, are caused by anoxemia and to what extent by other factors is not known

5 In a considerable number of pneumonic patients with abnormally low arterial oxygen saturation treatment with oxygen inhalation has

caused the abnormal oxygen unsaturation to disappear At the same time the anoxemic symptoms, especially the cyanosis, have either disappeared or decreased in intensity

In the British Physiological Society Barcroft, in 1920, in a lecture on anoxemia, said that "The physiology of today is the medicine of tomorrow" I consider this right only if between "today and tomorrow" intense and conscientious work is done to reconcile the synthetic researches of the physiologist with the analytic investigations performed at the bedside by the clinician

To do that not only in respect to physiology but also in respect to the other biological sciences is the main purpose of the theoretical medicine And the delineation of the problem is, to my mind, the main purpose of the teaching of theoretical medicine

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IMMUNIZATION AGAINST PNEUMONIA

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The high incidence rate of lobar pneumonia is a well known fact and remains a constant source of anxiety to the public health officer Obviously one of the most important sanitary problems of the day, the prevention of pneumonia is contingent on either the elimination of the virulent pneumococcus or the production of some form of artificial immunity against it At the present time the former procedure is entirely impractical Pneumonia does not lend itself readily to control by ordinary hygienic or sanitary measures The high incidence of pneumonia in cities is evidence that the pneumococcus thrives best when people live under crowded conditions Infection appears to be transmitted by direct or indirect contact, in many instances by the droplet method With the present tendency toward urbanization of our population there seems to be less and less hope of controlling pneumonia and other respiratory diseases by any of the hygienic measures now in vogue

From these considerations it would appear that for the present at least the only hope of preventing pneumonia lies in the adoption of some method of artificial immunization The simplest way to prevent pneumonia would be to prevent the common cold, for it is well recognized that pneumonia in man is usually secondary to a cold or a sore throat Unfortunately there is as yet no universally satisfactory method of preventing colds or sore throats, consequently until such a method is forthcoming, the attack upon pneumonia must be centred upon that disease itself

In view of the established value of vaccination against typhoid fever, it is really surprising that efforts to prevent pneumonia by means of vaccine were not undertaken earlier As a matter of fact it has been only in the last few years that any serious effort has been made along this line

Lobar pneumonia is an acute infectious disease, caused, in the great majority of cases, by the pneumococcus Approximately 95 per cent of all cases of true lobar pneumonia are of pneumococcal origin The streptococcus and Friedlander's bacillus are responsible for the few remaining cases In this article our attention will be confined to a consideration of pneumococcus immunity and its bearing on the prevention of pneumococcus pneumonia

IMMUNITY FOLLOWING LOBAR PNEUMONIA

It will not be out of place at this point to refer briefly to the four biological types of pneumococcus as first differentiated by Dochez and Gillespie (1). Morphologically and culturally these four types resemble one another closely but they display sharp and specific differences in respect to agglutinins, precipitins, and protective bodies It has, of course, been necessary to take these facts into consideration in an attempt to vaccinate against pneumococcus infection

When an animal or man survives an attack of pneumococcus pneumonia, a high degree of immunity against the homologous type of pneumococcus is readily demonstrable. The crisis itself is a striking expression of this immunity Furthermore, Dochez (2) has shown that the serum of patients convalescing from pneumonia contains protective substances against the homologous type of pneumococcus, and Blake (3) has demonstrated precipitins in the serum of cases of pneumonia that terminate favorably In addition to these clinical studies, accurate information on the subject of immunity following pneumonia has been obtained from experimental work on animals In some studies on experimental pneumonia conducted by Cecil and Blake (4) in 1920, these authors showed that in monkeys an attack of pneumococcus Type I pneumonia protects the animal completely against a second infection of the same type An attack of pneumococcus Type I pneumonia gave little if any protection against pneumococcus Type II pneumonia On the other hand, there appeared to be some cross-protection against pneumococcus Type III

During the last five years we have had opportunity to follow a number of patients through several attacks of pneumonia and in every instance recurring attacks have differed in type from the initial type.

The one exception appears to be in the case of pneumococcus Type III infections There are records of several patients who have had repeated attacks of pneumococcus type III pneumonia These attacks, however, rarely come oftener than once a year

If one can make any inferences from these observations they would be that (*a*) An attack of lobar pneumonia is followed by a high grade of immunity against the type of pneumococcus that excited the attack (*b*) This immunity is not permanent but probably lasts from six months to one year (*c*) There is a small amount of cross-immunity against other types of pneumococcus This, however is probably a variable and uncertain factor

ACTIVE IMMUNIZATION AGAINST THE PNEUMOCOCCUS

The earlier investigators were much interested in pneumococcus immunity A Frankel (5) made the important observation that rabbits inoculated with living, virulent pneumococci showed a high immunity if they recovered from the infection G and F Klemperer (6) induced a high degree of active immunity in rabbits against pneumococcus in several different ways They inoculated animals with heated pneumonic sputum, with pus from a pneumococcus empyema, and with cultures of pneumococcus which had been heated for one hour at 60°C Emmerick (7) injected rabbits with cultures which produced marked emaciation in the rabbits but did not kill them By this method he produced a very high immunity, the animals withstanding 20 to 30 cc of highly virulent culture intravenously These and other investigators have shown that an adequate immunity against pneumococcus infection can be developed in animals Neufeld (8) produced a high immunity in rabbits by subcutaneous and intravenous injections of killed pneumococci He found, however, that it was necessary to use a virulent culture Levy and Aoki (9) have immunized animals with pneumococci killed by phenol and also with sensitized pneumococci

Lister (10) found that specific agglutinins and opsonins could be produced in rabbits very easily by small doses of pneumococcus vaccine inoculated intravenously, and also, but with greater difficulty, if inoculated subcutaneously Such rabbits thus immunized were also able to resist an attack by virulent pneumococci when the latter

were inoculated in doses that would invariably kill a normal unvaccinated rabbit

Some interesting studies on pneumococcus immunity were carried out in 1917 by Solis-Cohen and Heist (11) Their observations were made on the whole blood of rabbits inoculated with killed pneumococci According to these experiments the whole blood of animals immunized against the pneumococcus possesses bactericidal power against pneumococci which is not possessed by the serum or defibrinated blood The bactericidal power thus induced was specific as regards type of pneumococcus

In addition to the immune bodies just referred to, the serum of an animal immunized against pneumococcus contains the so-called "protective substance" which has the power of protecting mice and other laboratory animals against lethal doses of virulent pneumococcus These various immune substances rarely make their appearance in the blood of the immunized animal before the seventh or eighth day after the first injection of vaccine

Larsen and Nelson (12) however, have recently found that if pneumococci are first treated with sodium ricinoleate, immune substances make their appearance much earlier in the blood of the vaccinated animal than when treated with ordinary vaccine

It is clear from this brief review of the literature that the pneumococcus differs in no way from other pathogenic bacteria in its capacity to stimulate active immunity in animals

The serum of animals vaccinated against pneumococcus not only develops bodies which provide the animal with an *active immunity* against the homologous type of pneumococcus, but the serum from these immunized animals is also capable of inducing *passive immunity* in normal unvaccinated animals against a fatal infection and even of curing them after infection has taken place, provided injection of the serum is not too long delayed This fact forms the basis for the modern serum treatment of lobar pneumonia in man

ACTIVE IMMUNIZATION AGAINST EXPERIMENTAL PNEUMONIA

While so many careful studies were being made of pneumococcus immunity, it was strange that little or no effort was being directed toward the study of active immunity against pneumonia itself The

reason for this failure was no doubt to be found in the difficulty which earlier investigators had in producing a satisfactory experimental pneumonia in animals

In 1904 (13), Wadsworth undertook to produce an active immunity against experimental pneumonia in rabbits Wadsworth injected rabbits intratracheally with virulent pneumococci and thereby excited a patchy form of pneumonia He then vaccinated normal rabbits with a saline suspension of pneumococci dissolved in rabbit bile The immunized rabbits were subsequently injected intratracheally with 1 cc of an extremely virulent culture of pneumococcus Of the 11 immunized animals none died, but a few were seriously ill for twenty-four to thirty-six hours When killed the vaccinated rabbits showed areas of diffuse consolidation involving considerable parts of the lung Of the 5 control rabbits, 3 died without lung lesions, the 2 others lived a few days longer and showed at autopsy small areas of consolidation in the lungs It is evident from these experiments that Wadsworth produced a partial immunity in rabbits against pneumococcus infection His infecting dose, however, was too large for the amount of immunity produced

In 1920 Blake and Cecil (14) perfected a technique for producing experimental lobar pneumonia in monkeys By injecting these animals intratracheally with minute doses of virulent pneumococci they were able to produce a typical lobar pneumonia differing in no respect clinically or pathologically from lobar pneumonia in man

Cecil and Blake (15) then undertook to study the effect of prophylactic vaccination against experimental pneumococcus pneumonia in monkeys In these experiments small doses of pneumococcus lipovaccine were used and each monkey received one inoculation subcutaneously By this method of vaccination partial immunity against the homologous type was established, but not enough to prevent mild attacks of pneumonia In a later study on monkeys Cecil and Blake found that the subcutaneous injection of a small dose of living, virulent pneumococci produced a high degree of active immunity sufficient to protect animals completely against experimental pneumonia of the homologous type Living cultures also stimulated a small amount of cross immunity against other types of pneumonia, which, however, varied considerably with individual animals Vaccination

with living, virulent pneumococci caused severe, at times fatal, reactions, while in other instances the reactions were very mild

It seemed quite possible that failure to obtain immunity with killed cultures of pneumococcus was due largely to inadequate dosage The writer, therefore, in collaboration with G I Steffen (16), continued the study of active immunity against pneumococcus pneumonia in monkeys, and found that the subcutaneous inoculation of monkeys with three large doses of pneumococcus Type I vaccine conferred upon them a complete immunity against experimental pneumococcus Type I pneumonia Cecil and Steffen also found that the intravenous inoculation of small doses of pneumococcus Type I vaccine conferred complete immunity against the homologous type of pneumonia In subsequent experiments (17) they found that as in the case of pneumococcus Type I, three subcutaneous injections of pneumococcus Type II vaccine would confer on monkeys a complete immunity against pneumococcus Type II pneumonia A similar protection could be bestowed on monkeys against pneumococcus Type IV pneumonia by three subcutaneous injections of a vaccine prepared from the same strain of pneumococcus The subcutaneous injection of monkeys with three doses of pneumococcus Type III vaccine conferred complete immunity against this type in only 50 per cent of cases (4 out of 8 monkeys vaccinated) In view of the difficulties associated with the vaccination of horses against pneumococcus Type III, the failure to obtain immunity against pneumococcus Type III in 100 per cent of monkeys was not surprising.

INTRATRACHEAL VACCINATION AGAINST PNEUMONIA

Several years ago it occurred to the writer that it might be feasible to establish an immunity against pneumonia by injecting pneumococcus vaccine directly into the trachea Such a procedure seemed entirely rational in consideration of the fact that in lobar pneumonia infection takes place through the trachea, and furthermore, that in the early stages pneumonia is a peribronchial infection In the first series of experiments carried out by Cecil and Steffen (18), ordinary pneumococcus vaccine was injected directly into the trachea of monkeys with a hypodermic syringe Each monkey received three injections of vaccine at intervals of five to seven days It was

found that monkeys could be readily immunized against the various types of pneumococcus by intratracheal injections of pneumococcus vaccine Indeed, the successful immunization of monkeys with three small intratracheal doses of vaccine afforded some evidence that pneumococcus immunity might be more readily induced by the intratracheal route than by the subcutaneous route In this connection it is interesting to note that monkeys vaccinated subcutaneously or intravenously against pneumococcus usually showed immune bodies in the circulating blood, while the monkeys vaccinated by the intratracheal route failed to show circulating antibodies This suggested that the immunity induced by the intratracheal injection of vaccine was in great part a cellular immunity

Cecil and Steffen also attempted to immunize monkeys against pneumonia by spraying them with pneumococcus vaccine Complete immunity against pneumonia was not obtained by this method of immunization The failure to obtain immunity by means of a spray may have been due to technical difficulties, but in this connection it will be appropriate to discuss the work of Stillman

Stillman (19) exposed mice to an atmosphere containing large numbers of virulent pneumococci He found that a high degree of active immunity could be produced in mice following repeated inhalations of living pneumococci, but a much less marked increase in resistance was afforded animals exposed to a spray of dead organisms In order to explain this acquired protection Stillman assumed that the inhaled pneumococci actually gained access to the body tissue, that is, they were not only implanted on the mucosa of the lower respiratory tract but actually penetrated the respiratory epithelium In sharp contrast to the favorable results obtained by the inhalation of living pneumococci, Stillman obtained only a slight immunity in mice by the repeated inhalation of killed organisms An interesting fact brought out by Stillman was that mice which had been immunized by inhalations of living or killed pneumococci, could be deprived of this immunity by subjecting them to alcoholic intoxication

ACTIVE IMMUNIZATION AGAINST PNEUMONIA IN MAN

Prior to 1911 no serious attempt had been made to vaccinate human beings against lobar pneumonia by means of pneumococcus vaccine

In 1911, however, the mortality from lobar pneumonia among the tropical natives in the South African Gold Mines became so high that the gold mining industry secured the services of Sir Almroth Wright to investigate the disease with a view to devising some method of diminishing the death rate

The investigations conducted by Wright and his co-workers extended from October 1911 throughout 1912 Wright (20) was chiefly interested in detecting differences in the serum of inoculated and uninoculated persons, particularly in respect to the opsonic index Wright was seeking some standard by which he could correlate the amount of immunity with the dose of pneumococcus vaccine and thus determine the most satisfactory prophylactic method of vaccination against pneumonia Wright's studies of the opsonic index after injections of pneumococcus vaccine failed to disclose any definite information Wright also found that the agglutination reactions were very irregular with the vast majority of strains of the pneumococcus

Wright next had recourse to inoculating several thousand miners with killed broth culture vaccine, setting aside control groups of natives for comparison The incidence of pneumonia among the vaccinated men six months to one year after inoculation was recorded and a similar record kept of the incidence of pneumonia among the unvaccinated miners These control groups, however, were not clearly defined and led to great difficulty in estimating the results of the various experimental inoculations

Wright's method of inoculation consisted at this time of the subcutaneous administration of one dose of pneumococcus vaccine containing 4 to 50 million killed bacteria Wright himself was convinced from his study that the incidence of pneumonia was considerably reduced during the first three months following inoculation Later reports, however, failed to establish the efficacy of his method of vaccination against pneumonia His failure was probably due to two things First, at the time his experiment was conducted, the various types of pneumococcus has not been dis[illegible]ished, second, the doses of vaccine employed were much too [illegible]

(22) then undertook an experimental study of prophylactic inoculation against the various types of pneumococci in animals and man He demonstrated that immunity could be produced in man against at least certain ones of these types either by subcutaneous or intravenous injection, more readily by the latter He found that subcutaneous inoculation of 40 billion cocci of the strains he employed caused little if any toxic reaction in the guinea pig, rabbit or man, and that even intravenous inoculations of several billion in the rabbit or in man gave rise to but slight toxic reaction

Experiments carried out by Lister at the South African Institute for Medical Research also proved beyond doubt that the blood serum of natives who had been inoculated with suitable doses of pneumococcus vaccine contained agglutinins and opsonins in substantial amounts The number of organisms necessary to produce these changes was however, far in excess of anything previously attempted, but, as in the case of rabbits, fewer organisms were necessary when the intravenous route was used than when the subcutaneous route was employed

In view of these favorable preliminary experiments Lister next undertook the prophylactic inoculation of large groups of miners against pneumonia Lister s nomenclature was somewhat different from that of Dochez and Gillespie He found that approximately 70 per cent of all cases of lobar pneumonia in South Africa were caused by infection with either group A, B or C groups B and C correspond ing to Types II and I respectively in Dochez and Gillespie's classification In Lister's first experiment 10 866 recruits were each inoculated three times with a vaccine containing equal parts of pneumococcus groups A, B and C In some instances Lister vaccinated natives intravenously, but later he adopted the subcutaneous method, giving each volunteer three subcutaneous injections consisting of 6 billion cocci of each group (a total of 18 billion) Subsequently he greatly reduced this dosage and gave three subcutaneous inoculations each injection consisting of 2 billion of each type Pneumococcus vaccine as at present prepared at the South African Institute for Medical Research contains 8 billion pneumococci per cubic centimeter comprising 8 different pneumococcus groups

The results of this experiment were very satisfactory Not a single case of lobar pneumonia due to groups A B or C occurred among the

vaccinated troops. Eighty-two cases due to other types occurred among the vaccinated troops During this time the case mortality among unvaccinated troops was 34.7 per cent. Lister contends that this fact namely, the alteration of a relative group prevalence by means of specific group inoculation is a more critical test of the efficacy of pneumonia prophylaxis than the simultaneous comparison of pneumonia rates in inoculated and uninoculated (control) groups when the comparison is based upon the erroneous assumption that all cases of disease due to the pneumococcus are bacteriologically indistinguishable. He emphasizes the probability that the protection of a considerable part of the community by inoculation lessens the number of carriers, and perhaps the virulence of the strains found in the community, and, hence, confers a definite benefit upon the uninoculated group which would affect the use of this group as controls in a statistical sense. Lister reported no unpleasant effects from the vaccine

Following this experiment on the Crown Mine, Lister carried out similar experiments at the Premier Diamond Mine in the Transvaal and at the DeBeers Diamond Mine at Kimberly. At the Premier Mine the death rate fell during the experimental period to about 1 per 1000 per annum, while at Kimberly in the DeBeers Mines the mortality from pneumonia was quickly reduced by more than 50 per cent. These experiments of Lister's were carried out during 1916 and 1917. During 1918 Lister arranged to inoculate all new native mine recruits with pneumococcus vaccine, three doses to be given at seven days interval. In Lister's (23) last report of March, 1924 he reviews the results obtained during the six years previous During this period there was a drop in the pneumonia death rate from 5 per 1000 in 1916 to 1 68 per 1000 in 1922. There was a slight increase in 1923 and 1924, but the figure remains in the neighborhood of 2 per 1000 of population According to Lister, infections due to the three great groups of pneumococci (A, B and C) are now relatively rare in South Africa and the great majority of the lobar pneumonias that do occur are due to strains other than those of the A, B and C groups

In South Africa the question has been raised, whether the improvement in the general hygiene at the Mines has not been the chief factor in the decreased pneumonia mortality. Lister points out, however, that the Premier Diamond Mine was about the worst mine in

South Africa as regards mortality for pneumonia, yet it was for the most part an open mine with abundant ventilation

In the winter of 1917–1918 lobar pneumonia became quite prevalent among the recruits who were in training at Camp Upton, N Y Bacteriological study of the cases showed that a high percentage were due to the pneumococcus In consideration of the favorable results achieved by Lister in South Africa, it seemed desirable to the writer to test the prophylactic value of pneumococcus vaccine on Camp Upton troops In collaboration with J H Austin (24) of the Rockefeller Institute an extensive field experiment was undertaken with a vaccine containing equal parts of pneumococcus Types I, II and III Vaccine against Type IV was obviously not feasible on account of its many varieties The vaccine was first tested on rabbits and on 42 adult volunteers It was found that an ordinary pneumococcus vaccine prepared from glucose broth cultures would stimulate agglutinins and protective substances within eight days after the first injection of vaccine Generally speaking, the larger the dosage of vaccine the better the response both in agglutinins and in protective bodies On the basis of these preliminary experiments, we adopted for the large experiment a total dosage of 6 to 9 billion cocci of each type administered in 3 or 4 doses at weekly intervals

Altogether, 12,519 men were vaccinated—about 40 per cent of the mean strength of the command A great majority of these received three or four inoculations Some, however, had only one or two The vaccine was given at five- to seven-day intervals, except in one organization where unavoidable circumstances necessitated a lapse of twenty days between the first and second inoculations In most cases the vaccine was administered in the arm

Reactions

The constitutional reactions to the injections of pneumococcus vaccine were usually negligible Out of the entire number vaccinated, only 25 men were sufficiently ill to remain in quarters or in the hospital for a short time This number would probably have been larger if the vaccination had been compulsory As it was, those who were upset by the first or second inoculation were usually not given the third or fourth The impression prevailed among the regimental

urgeons that the reactions to pneumococcus vaccination were milder han those to typhoid vaccination The troops, however, were in etter physical condition when they received the pneumococcus vac-ine than when the typhoid inoculations were given In those who eacted severely the symptoms simulated an attack of influenza The patient complained of general malaise, chilly sensations, fever nd muscular pains In addition, a certain number of those who eacted severely had symptoms referable to the upper respiratory ract, such as coryza, sore throat, cough, and pain in the chest A umber of those who were suffering from infections of the air passages t the time of inoculation, claimed that their symptoms were more narked after receiving the injection Constitutional reactions to neumococcus vaccination apparently develop more slowly than with yphoid inoculation, sometimes not appearing until twenty-four ours after the injection

The local reaction to pneumococcus vaccination differs little, as a ule, from that to typhoid vaccination At the point of inoculation n area of tenderness and induration develops, usually about 5 to 10 m in diameter The area of induration is nearly always oblong and xtends down the arm below the point of inoculation The axillary lands are sometimes swollen and tender The tenderness and welling at the point of inoculation rapidly decrease, however, and t the end of three or four days have usually disappeared

An unexpected and somewhat troublesome complication arose in onnection with the vaccination which at first gave some concern, his was the development of a certain number of small infiltrations at he site of inoculation They developed slowly, rarely coming to he stage of fluctuation before the sixth or seventh day after inocula-ion They were usually 2 or 3 cm in diameter, and in only one nstance extended down deeper than the subcutaneous tissue At irst they were looked upon as the result of careless technique, but epeated cultures showed them to be invariably sterile Some of them leveloped after the first inoculation, but a greater number followed the arger doses In a few cases an infiltration developed with each inocu-ation given. This would have probably happened more frequently had the vaccination not been discontinued in those who developed the ondition after the first or second dose At first the regimental sur-

geons made an incision, but later it was found that the infiltrations would progress favorably if left alone Altogether, 152 men developed the lesion (1 in every 82) The infiltrations were tender and painful in the early stage of their development but later became cold and painless

Results of vaccination

The vaccination of the troops was begun on February 4, 1918 The Division was transferred from Camp Upton about April 15, 1918 The following figures are based on the period extending from February 4 to April 15, about ten weeks The number of troops vaccinated was 12,519 The number of unvaccinated was approximately 19,481 The latter figure varied, of course, from day to day as new men came and others departed The vaccinated men were in stable organizations where the personnel underwent little change

During the ten weeks subsequent to vaccination no cases of pneumonia due to the three fixed types of pneumococcus occurred among the troops who had received two or more injections of vaccine One individual developed pneumococcus Type I pneumonia twenty-four hours after the first injection of vaccine, that is, before any protection could have been induced by the vaccine In the control group of approximately 20,000 unvaccinated men, there were 26 cases of pneumococcus Types I, II and III pneumonia during the same period Strangely enough, the incidence of pneumococcus Type IV pneumonia and of streptococcus pneumonia was also much lower among the vaccinated troops than among the unvaccinated There were 9 Type IV pneumonias and 7 streptococcus pneumonias among the vaccinated troops, whereas, among the unvaccinated men there were 33 type IV pneumonias and 106 streptococcus pneumonias The case mortality rate among the vaccinated was only 11 7 per cent, while that for the unvaccinated was 28 per cent

The results obtained by Cecil and Austin at Camp Upton were so promising that further tests with pneumococcus vaccine were undertaken the following winter

Cecil and Vaughan (25) conducted a second field experiment with pneumococcus vaccine at Camp Wheeler, Georgia On this occasion 13,460 men, about 80 per cent of the entire camp strength were

vaccinated against pneumonia with a pneumococcus vaccine containing 10 billion each of pneumococcus Types I, II and III in each cubic centimeter of vaccine In this experiment, however, the pneumococci were suspended in cottonseed oil instead of the usual salt solution Each soldier received a single injection subcutaneously The dose was 1 cc of the lipovaccine, equivalent to 30 billion pneumococci Conditions at Camp Wheeler were not nearly so favorable for testing the value of pneumococcus vaccine as they had been at Camp Upton The pandemic of influenza swept over the camp in the midst of the experiment and, because of the lowered resistance which the influenza virus induced, a certain amount of pneumonia of all types developed among the vaccinated men Furthermore, the pneumonia, which accompanied the influenza epidemic was due in great part to Type IV pneumococcus and streptococcus, neither of which organisms had been included in the vaccine The results obtained at Camp Wheeler, while not so successful as thoe at Camp Upton, were, nevertheless, quite encouraging. Four-fifths of the population were vaccinated, but almost as many cases of pneumonia developed among the unvaccinated one-fifth as occurred among the entire vaccinated four-fifths of camp Reckoning from one week after vaccination, the time when the individual's immunity begins to develop, only 8 cases of Types I, II and III pneumonia occurred among the vaccinated men and all those were secondary to severe attacks of influenza Using the same standard, 124 cases of Type IV pneumonia developed among the vaccinated troops and 103 of these were secondary to influenza. Reckoning from the day of vaccination, there were 33 cases of pneumococcus Types I, II and III pneumonia among the vaccinated four-fifths of the camp and 42 cases of pneumonia of these Types among the unvaccinated one-fifth of the camp The death rate for 155 cases of pneumonia, including all types that developed among vaccinated troops one week or more after vaccination, was only 12 2 per cent, whereas the death rate for 327 cases of all types that occurred among unvaccinated troops was 22 3 per cent

The author believes that even better results would have been obtained at Camp Wheeler, if a saline vaccine similar to that used at Camp Upton had been employed instead of the lipovaccine Experiments on animals have conclusively shown that bacteria suspended in

oil do not possess as potent an antigenic capacity as when suspended in salt solution In fact, lipovaccine has so many disadvantages that at the present time the Hygienic Laboratory of the United States Public Health Service will not issue licenses for its manufacture

During the winter of 1918–1919 pneumococcus vaccine was used extensively in the United States Army, both in the training camps and in the American Expeditionary Forces The following memorandum from the Surgeon General's Office in Washington is quoted from the official report of the Camp Surgeon at Camp Taylor, Kentucky (26)

January 28, 1919

Our records show that of the 4754 men who took pneumonia vaccine only 1 case of pneumonia has developed, while in the rest of the camp there have been over 80 cases These figures require no further elaboration and it is recommended that the inoculation be made compulsory

TABLE 1

Results of vaccination against pneumonia

	NUMBER OF MEN		NUMBER OF PNEUMONIA CASES		DEATHS	
	Vaccinated	Not vaccinated	Vaccinated	Not vaccinated	Vaccinated	Not vaccinated
	45,849	49,463	38	83	5	11
Rate per 100,000			83 5	168	10 8	22 5

Another memorandum was submitted to the Surgeon Generals' Office in April, 1919, by Major Fred M Meader, Medical Corps, showing the results of vaccination against pneumonia in Base Section No 2, A E F In table 1, cases were not counted unless they had developed seven days after vaccination It will be seen from this table that both the incidence-rate and the death-rate were twice as high in the unvaccinated as in the vaccinated series

In 1919, Major Borel (27) of the French Medical Corps made a favorable report on the use of pneumococcus vaccine among the colored troops in the French Army It seems that these troops, coming as they did from tropical colonies, were very susceptible to pneumonia when they reached France In one experiment three battalions were vaccinated and three others were used as a control

The vaccine was composed of killed pneumococci suspended in normal salt solution in a concentration of 4 billion bacteria per cubic centimeter. The doses used were (*a*) 0 5 cc (2 billion pneumococci), (*b*) 1 cc (4 billion pneumococci) eight days after the first injection No reaction, either general or local, was observed among those vaccinated The results obtained in this first experiment were very promising, although the various types of pneumococci were not contained in the vaccine

In a second experiment conducted by Major Borel, a pneumococcus vaccine composed of several types was prepared by Professor Nicolle at the Pasteur Institute, and 300 Sengalese were vaccinated with 3 subcutaneous injections (total—28 billion pneumococci) and 300 in the same organization were reserved for controls The result was 1 mild case of pneumonia and no deaths among the 300 vaccinated,

TABLE 2

Incidence-rate per 1000 persons

GROUPS	TOTAL NUMBER	CASES OF PNEUMONIA	DEATHS
Vaccinated 3 times	8,306	1 0	0 5
Not vaccinated	9,388	12 0	5 5

16 severe cases of pneumonia with 4 deaths among the unvaccinated controls The troops were under observation two months after inoculation The author concludes that pneumococcus vaccine is of great value and that its use should be continued

Rosenow and Sturdivant (29) vaccinated 8306 inmates of institutions with a mixed vaccine consisting of pneumococci of the four types, hemolytic streptococcus, streptococcus viridans, and staphylococcus aureus In the same experiment 9388 persons were not vaccinated and served as a control Table 2 shows the results obtained It will be seen from these figures that both the incidence-rate and the death-rate were materially decreased in the vaccinated series

Von Sholly and Park (30) vaccinated 1536 persons in the employ of the Metropolitan Life Insurance Company with a mixed vaccine directed primarily against the milder respiratory infections A control of 3025 persons remained unvaccinated This vaccine had

practically no effect on the incidence of influenza and colds, the rate remaining about the same in both groups The vaccine contained pneumococci of the three fixed types, streptococci, and influenza bacilli The interesting feature of this experiment was that only one case of pneumonia developed among the 1536 vaccinated employees, while 11 cases, or five times as many, occurred among the unvaccinated controls

The only report on pneumococcus vaccine which has not been entirely favorable is that of McCoy, Hasseltine, Wadsworth and Kirkbride (31) These investigators studied the value of prophylactic vaccination against pneumonia among the inmates of certain New York institutions The vaccine used was a lipovaccine containing approximately 10 billion each of pneumococcus Types I, II and III A single dose of 1 cc was administered subcutaneously to 17,752 patients, while 18,595 remained unvaccinated The patients were under observation approximately two years or rather during two pneumonia seasons Among the vaccinated half, 253 cases of pneumonia developed, while 340 cases occurred among the uninoculated Of these cases only 122 in the vaccinated series and 186 in the control series were studied bacteriologically An analysis of the bacteriological findings in this study is very interesting and possibly explains why more convincing results were not obtained In the control series, only 23 6 per cent of typed cases fell into the groups of pneumococci (Types I, II and III) against which the vaccine had been directed, 76 4 per cent of the cases being caused by other organisms—pneumococcus Type IV, streptococcus, B influenza, Friedlander's bacillus, etc In the vaccinated series, only 18 per cent of the classified cases fell into the fixed types of pneumococcus It should be noted further that, of the 22 cases of fixed type pneumonia that developed among the vaccinated patients, 16 were classified under pneumococcus Type III the group which in civil life is most rarely encountered and against which, in animal experiments, it is most difficult to immunize After making all allowances, however it is noteworthy that, among 17,752 persons vaccinated against pneumonia and under observation for two years thereafter, there occurred only 1 case of pneumococcus Type I pneumonia and only two cases of pneumococcus Type II pneumonia! Of course there may have been a

few more of these types among the unclassified cases It is a well-known fact that the pneumonia which occurs in institutions for the insane, or, for that matter, in any institution, is nearly always of the bronchial type and presumably of streptococcus or pneumococcus Type IV origin At Saranac Lake lobar pneumonia is practically never encountered in sanitariums for tuberculous patients My criticism of this experiment, then, is that it was not a fair test for pneumococcus vaccine in that the vaccine was not directed against the type of pneumonia which was prevalent in these institutions.

Preparation of pneumococcus vaccine

The original pneumococcus vaccine used by Wright and by Lister was prepared from cultures grown on plain or glucose agar Most of the pneumococcus vaccine manufactured in this country has been prepared from broth cultures

Pneumococci are cultivated for eighteen to twenty-four hours on plain or glucose broth The culture is then centrifuged, and the sediment of bacteria suspended in normal salt solution Finally the saline solution is heated at 55°C for one-half hour to kill the pneumococci, and the vaccine standardized by the Wright method or by means of a nephelometer Cultures are taken to test the sterility of the vaccine and tricresol is added to a concentration of 0 3 per cent as a preservative

Methods of administering pneumococcus vaccine

Pneumococcus vaccine is almost always administered subcutaneously The proper method of giving the vaccine is to pinch up the skin and insert the needle well under the dermis Intracutaneous injections excite severe local reactions Lister experimented with intravenous injections of pneumococcus vaccine and the writer has tried it on several occasions. The intravenous method would be the method of choice were it not for the danger of exciting constitutional reactions The immune response to intravenous vaccination is quicker and more marked than that to subcutaneous injections The intravenous injections, however, are apt to excite the so-called non-specific protein reaction (chill, fever, leuckocytosis, etc) similar to that following the intravenous injection of typhoid vaccine As a

matter of fact, pneumococcus vaccine when injected intravenously is much less likely to induce a foreign protein reaction than the same dose of typhoid vaccine The writer, however, does not recommend the intravenous method of vaccination for the reason that adequate immunity can be obtained by subcutaneous injections

Dosage

For therapeutic purposes, pneumococcus vaccine is administered in doses varying from 10 million to 1 billion pneumococci or even more For prophylaxis, much larger doses are used The vaccine as prepared at the United States Army Medical School contained equal parts of pneumococcus Types I, II and III In the United States Army, 3 to 9 billion was the dose of saline vaccine, 30 to 40 billion of the lipovaccine In the case of saline vaccine, three injections were given at seven day intervals, the first dose, 3 billion, the second, 6 billion, and the third, 9 billion In civil life we have used a vaccine consisting of equal parts of pneumococcus Types I, II and III, suspended in salt solution, so that 1 cc contains a total of 9 billion killed bacteria Three injections are given, separated by intervals of one week, as follows

First injection	0 3 cc (3 billion)
Second injection	0 6 cc (6 billion)
Third injection	1 0 cc (9 billion)

Reactions

Both the local and general reactions to pneumococcus vaccine vary greatly in different individuals In general it may be said that the smaller the dose the milder the reaction It is therefore desirable, if circumstances permit, to divide the total dosage (18 billion) into 4 or 5 inoculations The writer wishes to emphasize once more, that within certain limits, the larger the total dose of vaccine administered the higher will be the immunity conferred

In general it may be said that the local reaction to pneumococcus vaccine is similar to that following the subcutaneous injection of typhoid vaccine Within twenty-four hours after inoculation an area of redness and induration appears at the site of injection which is usually 2 or 3 cm in diameter but may be larger The small sterile

abscesses previously described, which occasionally occur at the site of injection, are seen only in cases where large doses of vaccine have been used These infiltrations are uncomfortable but are not serious, and disappear spontaneously The constitutional reaction to pneumococcus vaccine is usually insignificant In many cases it is entirely absent In a small percentage of cases vaccination is followed by headache or backache, general malaise, chilly sensations and a rise in temperature These symptoms, however, are generally of short duration

Indications for pneumococcus vaccine

Pneumonia is a very prevalent disease, but hardly prevalent enough at present to justify a general and indiscriminate use of pneumococcus vaccine There would seem to be two particular indications, however, for the use of pneumococcus vaccine, (*a*) For individuals who are very susceptible to pneumonia and suffer from repeated attacks of the disease During the past six or seven years the writer has vaccinated a number of such persons and in no instance has the vaccine failed to give complete protection against a recurrence of the disease (*b*) Pneumococcus vaccine is particularly valuable in the case of recruits in time of war In previous wars intestinal infections were the chief menace to health In the late war, pneumonia was the most serious of all the infections and caused the greatest loss of life. Pneumococcus vaccine is clearly indicated in the case of green recruits who are suddenly mustered into training camps where they are subject to contact with pneumococcus carriers, exposure to inclement weather and the lowering of resistance which comes with fatigue and overexertion Pneumococcus vaccine could probably be used with benefit in the case of industrial workers, such as day laborers, truck drivers, chauffeurs, firemen, and policemen, who are constantly exposed to wet and cold The practical difficulty which one encounters here is the strong objection which these people have to taking the vaccine on account of the reactions

The advisability of vaccinating nurses, physicians and relatives who come in contact with a patient with pneumonia is a question which has not received much attention The writer has seen a number of cases of contact infection in the same family Pneumonia is not

looked upon as a contagious disease, but occasionally it becomes so Once we obtain a potent vaccine which excites little or no reaction it may be worth while to vaccinate everyone who comes in contact with a pneumococcal infection

Contra-indications

Pneumococcus vaccine should not be administered during an acute infection, or to patients with pulmonary tuberculosis It should not be administered in large doses to patients with chronic cardiac or renal diseases, or to pregnant women It should preferably not be administered during menstruation

Intratracheal vaccination against pneumonia

On account of the severe reaction sometimes produced by pneumococcus vaccine when injected subcutaneously, it is clear that improvements in the method of preparation and in the method of administration will have to be forthcoming before active immunization against pneumonia will become popular in civil life With regard to modifications in the method of administration it is quite possible that a satisfactory immunity against pneumonia could be obtained in man as in monkeys by injecting the vaccine directly into the trachea

In a preceding section the writer has reported some experiments which throw some light on this subject In these experiments it was found that the intratracheal injection of pneumococcus vaccine affords just as satisfactory an immunity against pneumonia as that induced by subcutaneous or intravenous injections At the same time an attempt was made to immunize monkeys against pneumonia by spraying them with pneumococcus vaccine Complete immunity against pneumonia was not obtained by this method, probably because the monkeys offered a great deal of resistance to the treatment and because the spray was not continued over a sufficiently long period of time It is possible that the daily inhalation of a pneumococcus vaccine spray would prevent completely the severer forms of lobar pneumonia in man The immunity established against pneumococcus by vaccination appears to be of rather short duration, but with an atomizer the spray could be used frequently during the winter months and permanent immunity maintained in this way

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Immunization against pneumonia with derivatives from the pneumococcus

During recent years a number of attempts have been made to isolate a pneumococcus antigen with the hope that some method might be devised for eliminating the toxic fraction of the pneumococcus and provide a more potent agent for vaccinating against pneumonia in man. Warden (32), for example, claimed that the lipoidal fractions from pneumococci contained the antigenic principle, and that with this principle he could successfully immunize animals against pneumococcus infection Perlzweig and Steffen (33) were unable to verify the findings of Warden They studied the antigenic value of various derivatives from the pneumococcus and found that the immunizing antigen could be isolated from the three fixed types of pneumococcus by comparatively simple chemical methods According to these workers, this antigen in its purified form contains only a trace of nitrogen and is probably non-protein in nature Furthermore, they found that pneumococcus antigen is resistant to prolonged autolysis, and to tryptic digestion, and can be recovered from the soluble portions of digests of either the intact bacteria or the bacterial protein. The antigen may be isolated from the 3 fixed types of pneumococcus by tryptic digestion of the pneumococci and extraction of the digest with 70 to 90 per cent alcohol The antigen is not soluble in absolute alcohol, nor is it soluble in ether or the other lipoidal solvents Perlzweig and Steffen concluded that pneumococcus antigen is non-lipoidal, that it probably adheres to the protein fraction in a loose chemical or physical union rather than representing a protein complex of large molecular size, as shown by its solubility in alcohol, its thermostability and its resistance to proteolytic digestion These investigators found that purified pneumococcus antigen solutions are non-toxic for mice, and that ordinary laboratory animals can be actively immunized by them More recently human subjects have been successfully vaccinated with this antigen It excites little or no local reaction and the immune response is excellent.

Ferry and Fisher (34) have also studied the immunizing properties of pneumococcus antigen prepared after various methods. They found that antigen, apparently protein free, gave evidence of excellent

Pneumococcus vaccine when given in adequate doses confers nunity against pneumococcus pneumonia in man The evidence favor of such immunity rests on statistical studies and on the ionstration of immune bodies in the blood of those vaccinated

The chief indications for the use of pneumococcus vaccine at present time are *First*, to prevent recurrence in individuals who highly susceptible to repeated attacks of lobar pneumonia *nd*, for the prevention of outbreaks of pneumonia among fresh uits in training camps

There is reason to believe that the recent isolation of certain vatives of the pneumococcus which contain the antigenic prin- e will eliminate the undesirable features of vaccination against umonia and thereby render the procedure more practical in civil

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